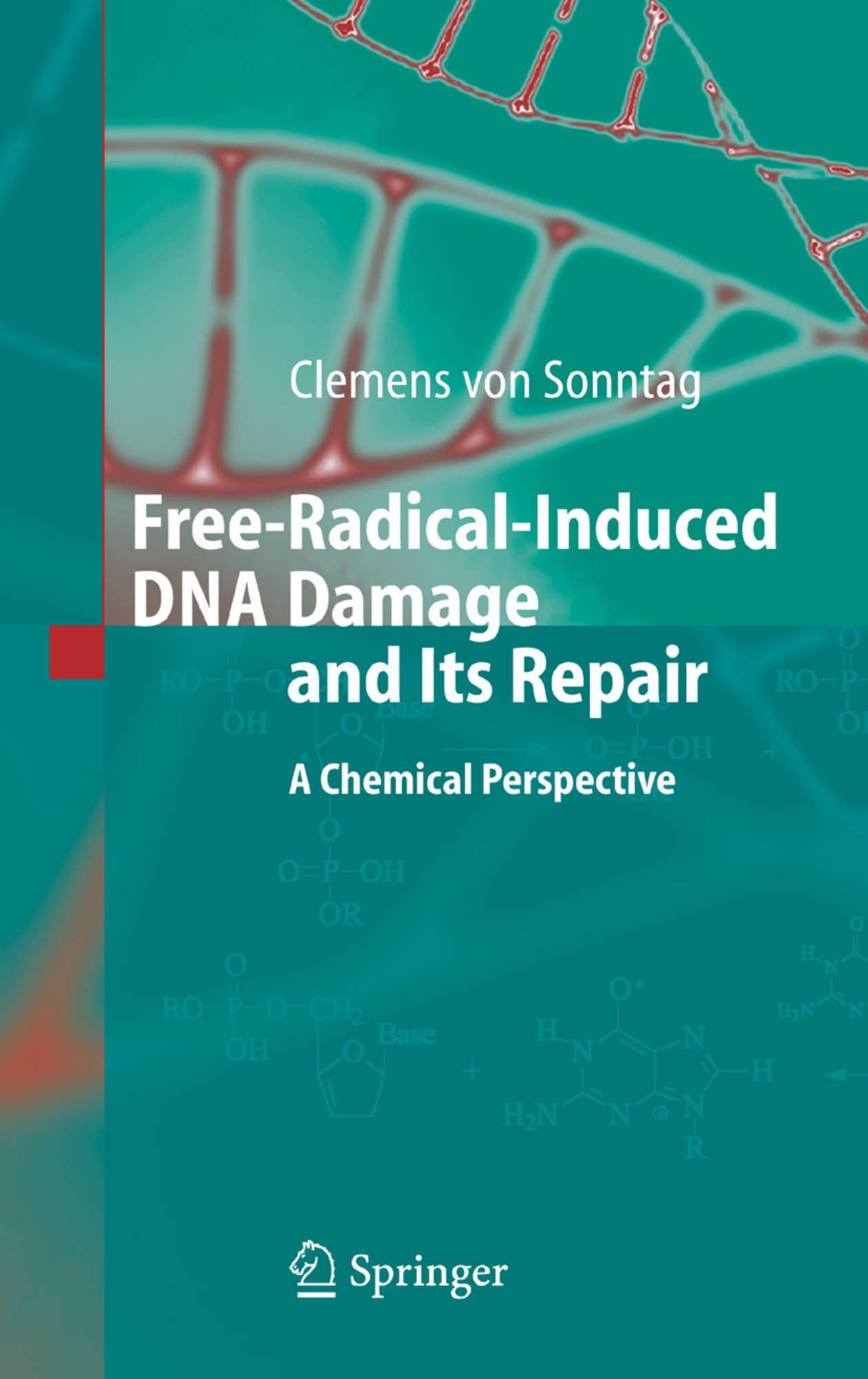
Clemens von Sonntag

Free-Radical-Induced DNA Damage and Its Repair

A Chemical Perspective



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With 13 Figures and 100 Tables

Springer

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Preface

The free-radical chemistry of DNA had been discussed in some detail in 1987 in my book *The Chemical Basis of Radiation Biology*. Obviously, the more recent developments and the concomitant higher level of understanding of mechanistic details are missing. Moreover, in the living cell, free-radical DNA damage is not only induced by ionizing radiation, but free-radical-induced DNA damage is a much more general phenomenon. It was, therefore, felt that it is now timely to review our present knowledge of free-radical-induced DNA damage induced by all conceivable free-radical-generating sources. Originally, it had been thought to include also a very important aspect, the repair of DNA damage by the cell's various repair enzymes. Kevin Prise (Cancer Campaign, Gray Laboratory, London) was so kind to agree to write this part. However, an adequate description of this strongly expanding area would have exceeded the allocated space by much, and this section had to be omitted.

The directors of the Max-Planck-Institut für Strahlenchemie (now MPI für Bioanorganische Chemie), Karl Wieghardt and Wolfgang Lubitz, kindly allowed me to continue to use its facilities after my retirement in 2001. Notably, our librarian, Mrs. Jutta Theurich, and her right-hand help, Mrs. Rosemarie Schrader, were most helpful in getting hold of the literature. I thank them very much. Without their constant help, this would have been very difficult indeed.

After my retirement at the MPI, Reiner Mehnert and recently also his successor, Michael Buchmeiser, gave me the most enjoyable opportunity to continue with research at the Leibniz-Institut für Oberflächenmodifizierung (IOM) at Leipzig and to finalize projects that were left over from my time at the MPI. Working continuously on the present book prevented me, of course, from fully devoting my time to ongoing IOM projects, and I am more than thankful that this was possible.

When talking about research, I must mention Mrs. Rita Wagner, a superb technician that looked after my laboratory and the younger Ph.D. students for about 25 years. Some of her fine work is referred to here. I have often been envied for being so lucky to have had her. It was also very fortunate that Heinz-Peter Schuchmann joined my group soon after my start at the MPI. His competence complemented mine extremely well, and this allowed us to carry out various projects that would not have been possible for either of us on our own. When Dietrich Schulte-Frohlinde was director, DNA free-radical research was the main research topic of the MPI, and our groups collaborated very closely for many years. With Peter Schuchmann and Dietrich Schulte-Frohlinde common interests beyond science led to a continuing friendship.

I flooded a number of good friends with questions, and they all were most helpful by answering them in a continuous exchange of e-mails. I cannot mention all of them, but I must mention, in alphabetical order, David Close, Gábor Merényi, Sergej Naumov, Herwig Paretzke, Kevin Prise, Peter Schuchmann, Michael Sevilla, Melanie Spotheim-Maurizot and Peter Wardman.

I did not realize that writing this book was to take much more time than I had anticipated from the experience with the preceding book. My charming wife, Ilsabe, took this with some amusement and without grumbling. Being herself a professional painter, she knew all too well that unless one is fully immersed in a project one will not achieve much. I am very grateful for her patience and encouragement.

Clemens von Sonntag
Mülheim and Leipzig, October 2005

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Abbreviations

A	Adenine (in DNA or oligonucleotide)
$A^{\bullet}$	Adenyl radical (in general; also with respect to dAdo, Ado and DNA)
$A^{\bullet+}$	Adenine radical cation (in general; also with respect to dAdo, Ado and DNA)
ABTS	2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonate)
Ade	Adenine
Ado	Adenosine
ADP	Adenosine-5′-diphosphate
AIM	Atoms-in-Molecules theory
AMP	Adenosine-2′-phosphate
AP	Apurinic/apyrimidinic site; abasic site
Ape	Apurinic/apyrimidinic endonuclease
AQS	*N*-(3-Aminopropyl)-2-anthraquinonesulfonamide hydrochloride
27AQS2	*N*,*N*,*N*′,*N*′-Tetrakis-(3-aminopropyl)-2,7-anthraquinonedisulfonamide hydrochloride
AQSO	Anthraquinone-2-sulfonic acid
ARB	Alkali revealed breaks
A^T	Tandem lesion, where *C*(8) of A is bound to the methyl group of T
BDE	Bond dissociation energy
BER	Base excision repair
BLM	Bleomycin
bp	Base pair
8BrGuo	8-Bromoguanosine
5BrUra	5-Bromouracil
$5BrUra^{\bullet-}$	5-Bromouracil radical anion
BSO	D,L-Butione-*S*,*R*-sulfoxime
C	Cytosine (in DNA or oligonucleotide)
C4-AP	*C*(4′)-Keto abasic site
cA	5′,8-Cyclo-2′-deoxyadenosine (also in AMP, dAMP and DNA)
Cg	5,6-Dihydroxy-5,6-dihydrocytosine, cytosine glycol (in general; also with respect to dCyd, Cyd and DNA)

cG	5′,8-Cyclo-2′-deoxyguanosine (also in GMP, dGMP and DNA)
5′-CHO-dAdo	2′-Deoxyadenosine-5′-aldehyde
CIDNP	Chemically induced dynamic nuclear polarization
CMP	Cytidine-5′-phosphate
Cyd	Cytidine
Cyt	Cytosine
dAdo	2′-Deoxyadenosine
dAMP	2′-Deoxyadenosine-5′-phosphate
dCMP	2′-Deoxycytidine-5′-phosphate
DEM	Diethylmaleate
DEPMPO	5-Diethoxyphosphoryl-5-methyl-3,4-dihydro-5*H*-pyrrole-*N*-oxide
DFT	Density Functional Theory
dGMP	2′-Deoxyguanosine-5′-phosphate
dGuo	2′-Deoxyguanosine
DIAMIDE	Diazenedicarboxylic acid bis(*N*,*N*′)-ethylmaleimide
dIMP	2′-Deoxy-inosine-5′-phosphate
DK	Dielectric constant
DMF	Dose modifying factor
DMPO	5,5′-Dimethyl pyrrolidine-1-oxide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
2-dR	Apurinic/apyrimidinic site containing a 2-deoxyribose unit
2-dRL	Apurinic/apyrimidinic site containing a 2-deoxyribonolactone unit
ds	Double-stranded
DSB	Double-strand break
dsDNA	Double-stranded DNA
DTPA	Diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*‴-pentaacetate
DTT	1,4-Dithiothreitol
dUMP	2′-Deoxyuridine-5′-phosphate
dUrd	2′-Deoxyuridine
e_{aq}^-	Hydrated electron
e-beam	Electron beam
EDTA	Ethylenediaminetetraacetic acid
EndoIII	Endonuclease III
EPR	Electron paramagnetic resonance
ET	Electron transfer
EtOH	Ethanol
ExoIII	5′ AP endonuclease exonuclease III
FAPY-A	4,6-Diamino-5-formamidopyrimidine (in general; also with respect to dAdo, Ado and DNA)
FAPY-G	2,6-Diamino-4-hydoxy-5-formamidopyrimidine (in general; also with respect to dGuo, Guo and DNA)

Fo	Formamido lesion within ODNs and DNA
FordUrd	5-Formyl-2′-deoxyuridine
5-FordUrd	5-Formyl-2′-deoxyuridine
5ForU	5-Formyl-2′-deoxyuridine in DNA
ForUra	5-Formyluracil
Fpg	Formamidopyrimidine-DNA-*N*-glycosylase
G	Guanine (in DNA or ODNs)
$G^{\bullet}$	Guanyl radical (in general; also with respect to dGuo, Guo and DNA)
$G^{\bullet+}$	Guanine radical cation (in general; also with respect to dGuo, Guo and DNA)
GG	Guanine doublets in DNA
GGG	Guanine triplets in DNA
GMP	Guanosine-5′-phosphate
GSH	Glutathione
Gua	Guanine
Guo	Guanosine
G^T	Tandem lesion, where *C*(8) of G is bound to the methyl group of T
H_2Thd	5,6-Dihydrothymidine
H_2Thy	5,6-Dihydrothymine
Hepes	*N*-(2-Hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid
HMdUrd	5-Hydroxymethyl-2′-deoxyuridine
HMUra	5-Hydroxymethyluracil
5HmU	5-Hydroxymethyl-2′-deoxyuridine in DNA
4-HO-8-oxo-G	4-Hydroxy-8-oxo-guanine (in general; also with respect to dGuo, Guo and DNA)
HPLC	High-pressure liquid chromatography
5,6H_2T	5,6-Dihydrothymine in DNA
5,6H_2Thy	5,6-Dihydrothymine
imidazolone	2-Amino-5-[(2-deoxy-β-D-pentafuranosyl)-amino]-4*H*-imidazol-4-one
IMP	Inosine-5′-phosphate
Iz	Imidazolone = 2-amino-5-[(2-deoxy-β-D-pentafuranosyl)-amino]-4*H*-imidazol-4-one
KIE	Kinetic isotope effect
LD_{50}	Dose required to reduce a cell population to 50% (LD = **l**ethal **d**ose)
LET	Linear energy transfer
LMDS	Locally multiply damaged sites
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
mC	5-Methylcytosine (in DNA)
menadione	2-Methyl-1,4-naphthoquinone
5MeCyt	5-Methylcytosine
5MedCyd	5-Methyl-2′-deoxycytidine

6MeUra	6-Methyluracil
5-MF	5-Methylene-furanone
MeOH	Methanol
1,3Me_2Ura	1,3-Dimethyluracil
1,3,6Me_3Ura	1,3,6-Trimethyluracil
Mn-TMPyP	*meso*-Tetrakis(*N*-methyl-pyridinium-4-yl)porphyrinatomanganese(II)-pentaacetate
MS	Mass spectrometry
mt-DNA	Mitochondrial DNA
NCS	Neocarzinostatin
NEM	*N*-Ethylmaleimide
NER	Nucleotide excision repair
NF^-	Nitroform anion
NHE	Normal hydrogen electrode
nitro blue tetrazolium	2,2′-Di-*p*-nitrophenyl-5,5′-diphenyl-3,3′-(3,3′-dimethoxy-4,4′-diphenylene)ditetrazolium dichloride
NPSH	Non-protein sulfhydryl
NTA	Nitrilotriacetic acid
Nth	*E. coli* endonuclease III
ODN	Oligodeoxynucleotide
OER	Oxygen enhancement ratio
5OHC	5-Hydroxycytosine in DNA
$(OH)_2$dUrd	5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine
5OHdUrd	5-Hydroxy-2′-deoxyuridine
5OH5,6H_2T	5-Hydroxy-5,6-dihydrothymine in DNA
5OHH_2Thy	5-Hydroxy-5,6-dihydrothymine
6OHH_2Thy	6-Hydroxy-5,6-dihydrothymine
5OHH_2Ura	5-Hydroxy-5,6-dihydrouracil
6OHH_2Ura	6-Hydroxy-5,6-dihydrouracil (also in poly(U))
5OHHyd	5-Hydroxyhydantoin
5OH5MeHyd	5-Hydroxy-5-methylhydantoin
5OHU	5-Hydroxy-2′-deoxyuridine in DNA
$OPCu^+$	(1,10-Phenanthroline)-Cu(I)
$(OP)_2Cu^+$	Bis(1,10-phenanthroline)-Cu(I)
oxazolone	2,2-Diamino-4-[(2-deoxy-β-D-pentofuranosyl)amino]-2,5-dihydrooxazol-5-one
8-oxo-A	8-Hydroxy-7,8-dehydroadenine (in general; also with respect to dAdo, Ado and DNA)
8-oxo-G	8-Hydroxy-7,8-dehydroguanine (in general; also with respect to dGuo, Guo and DNA)
8-oxo-G/Fo	Tandem lesion: 8-hydroxy-7,8-dehydro-2′-deoxyguanosyl-(3′→5′)-*N*-(2-deoxy-β-D-erythro-pentofuranosyl)-formamide
PICA	Polyamine-induced compaction and aggregation
PNAP	*p*-Nitroacetophenone
poly(A)	Poly(adenylic acid)
poly(C)	Poly(cytidylic acid)

poly(G)	Poly(guanylic acid)
poly(U)	Poly(uridylic acid)
2-PrOH	2-Propanol
PSET	Photoinduced single-electron transfer
PT	Proton transfer
PT–ET	Proton-coupled electron transfer
RBE	Relative biological efficiency
RNA	Ribonucleic acid
RSH	Any thiol
SASP	Small acid-soluble protein
SCE	Sister chromatid exchange
SIM	Single-ion monitoring
SOD	Superoxide dismutase
Sp	Spiroiminodihydantoin
SR4233	See tirapazamine
ss	Single-stranded
SSB	Single-strand break
ssDNA	Single-stranded DNA
T	Thymine (in DNA and oligonucleotides)
T^A	Tandem lesion, where *C*(8) of A is bound to the methyl group of T
T^G	Tandem lesion, where *C*(8) of G is bound to the methyl group of T
TAN	Triacetoneamine-*N*-oxyl
TBA	2-Thiobarbituric acid
*t*BuOH	Tertiary butyl alcohol
Tg	5,6-Dihydroxy-5,6-dihydrothymine (thymine glycol)
Thd	Thymidine
THF	Tetrahydrofuran
Thy	Thymine
tirapazamine	3-Amino-1,2,4-benzotriazine 1,2-dioxide
TLC	Thin layer chromatography
TMP	Thymidine-5′-phosphate
TMPD	*N*,*N*,*N*′,*N*′-Tetramethylphenylenediamine
TNM	Tetranitromethane
trans-resveratrol	3,5,4′-Trihydroxy-*trans*-stilbene
Tris	2-Amino-2-hydroxy-1,3-propanediol
TRLS	Time-resolved light-scattering
TRP	μ-[*meso*-5-5,10,15,20-Tetra(pyridyl)porphyrin]tetrakis[*bis*-(bipyridine)chloride ruthenium(II)]
U	Uracil (in RNA and oligonucleotides)
Ug	5,6-Dihydroxy-5,6-dihydrouracil, uracil glycol (also in nucleosides, nucleotides and DNA)
UMP	Uridine-5′-phosphate
Ura	Uracil
Ura glycol	5,6-Dihydroxy-5,6-dihydrouracil

Urd	Uridine
WIN 59075	See tirapazamine
WR-1065	2-(3-Aminopropylamino)ethylthiol
WR-2721	*S*-2-(3-Aminopropylamino)ethylphosphorothioic acid
Z	Oxazolone

Introduction

A series of non-connected facts are difficult to remember. The pre-war German chemistry textbooks that were still in common use in my student times contained such an agglomeration of facts. In contrast, the mechanistically oriented Anglo-Saxon textbooks were a pleasure to read. They taught us the underlying principles into which facts fitted quite smoothly. Ever since, I have had a foible for mechanisms, the little brother of the 'laws' that physicists enjoy in their profession. Mechanisms are only concepts, and we should not adhere to them without reservations, otherwise we get trapped by our prejudices. Our unjustified bias for a certain concept has been brought to the point by the German physicist and aphorist *Georg Christoph Lichtenberg* (1742-1799) in his '*Sudelbücher* (entry L 674)': "*Bei den meisten Menschen gründet sich der Unglaube in einer Sache auf den blinden Glauben in einer anderen*", that is, "with most people, the disbelief in something is based upon a blind belief in something else". The development of new concepts is a major contribution to progress in science, and work that leads to mechanistic concepts is presented here with some preference. However, according to *Alfred Popper*, mechanisms or any other concepts in science can never be proved; they can only be falsified. A mechanism may be called 'accepted' as long no experiment has been thought of and has been carried out that could falsify the present view. It is hoped that the mechanistic formulations given in this book will trigger further research by trying to falsify them and will thus lead to a much better understanding of free-radical-induced DNA damage and repair.

Mechanistic aspects can only be adequately dealt with when the complexity of the system is reduced to the essential, and the reader will see that there is a strong emphasis on DNA model systems such as the free-radical chemistry of nucleobases, nucleosides and nucleotides. Increasingly, compounds are synthesized, even up to the double-stranded oligonucleotide level, that allow the generation of a specific radical, e.g., by photolysis. This is an important breakthrough as far as mechanistic studies are concerned. Yet most of our present knowledge of the free-radical chemistry of DNA and its model systems has been obtained by radiation-chemical techniques (induced by attempts to improve radiotherapy and by radiation protection concerns). Obviously, the reactions of a given radical does not depend on its mode of generation, and it has been tried throughout the book to extract from these data the more general aspects of DNA free-radical chemistry rather than those particular to radiation-chemical effects. This, of course, had to lead to a suppression of some very fine radiation-chemical studies, notably in the area of low-temperature EPR. Moreover, some of the excellent biochemical studies such as details of the site-specificity of a given free-radical reaction did not find an adequate discussion. Space just did not permit this.

Some of the different chemistry of DNA as compared to its low-molecular-weight model compounds is due to the fact that DNA is a polymer, and some aspects of polymer free-radical chemistry are dealt with in a separate chapter. The special properties of dsDNA allow hole and electron transfer to trapping sites. This is an area that attracts very strong attention at present, and the level of understanding is already very high.

One way of oxidizing selectively the Gua moiety of DNA is the use of inorganic radicals having the right redox potential. A small chapter is devoted to such free-radical probes.

A number of anticancer drugs that kill cells by destroying their DNA via free radicals are used in the clinic. They show a most remarkable chemistry. Some of it is fairly well understood. Where important open questions remain, attention is drawn to these in order to elicit future studies that are urgently needed for a better understanding of the underlying mechanistic principles.

Free-radical-induced reproductive cell death is the basis of radiotherapy, and it is obvious that the main problem of this approach to fight cancer is to target the ionizing radiation to the tumor in order to prevent damage to healthy tissue. This is a most difficult if not impossible task. Radiation modifiers that sensitize the tumor cells and protect healthy tissue are considered for improving treatment regimes in radiotherapy, and the underlying mechanistic principles are addressed.

Free radicals, notably the superoxide radical, are by-products of the cellular metabolism and transition-metal ions seem to play a role in causing DNA damage in vivo. This may lead to mutations and eventually to cancer, and some of the phenomena of aging have also been attributed to free-radical-induced DNA damage. We are still far from understanding these reactions in sufficient detail, but the reader will find chapters on peroxyl radical chemistry and on some aspects of the involvement of transition-metal ions in free-radical reactions on which future work may be based.

Cells have two defense systems to cope with free-radical DNA damage that work on very different time scales: the fast 'chemical repair' by thiols that occurs at the stage of DNA free-radicals and the slow enzymatic repair that only sets in once the damage is fully set. The present book deals in some detail with the chemical repair. To discuss the even more important enzymatic repair would have exceeded the space allocated to this book, and enzymatic repair is only briefly touched on.

It is impossible in a book of a scope as wide as the present one to refer to all studies that may be relevant to a certain topic, but the many references that are given here will allow the reader to find an entry into the wider literature.

In science, there is a hierarchy of questions: (i) 'what', (ii) 'how', and (iii) 'why'. The report of a given fact, e.g., the determination of a series of products and their yields, only answers the question 'what'. Additional kinetic studies raise our level of understanding, as it answers the question 'how'. The ultimate scientific question, 'why', has as yet rarely been answered, but this level of knowledge is a prerequisite for being able to predict a certain reaction without too many flanking experiments. Thus, it will be one of the main goals of future research to strive for an in-depth theoretical understanding. This, of course, has to be based on our present (and future) experimental data, and it is one of the intentions of this book to provide the necessary information in a compact form.

DNA research is a very multidisciplinary field, with contributions by biologists, biochemists, chemists, physicists and theoreticians. Hence, the presentation given here should be at a level that it can be understood even when the educational background is not chemistry. In parts, this goal may not have been fully reached, but at other instances it may have led to some oversimplifications. I ask for apologies. Yet, in the 1920s, nuclear physicists enjoyed discussing that *Wahrheit* and *Klarheit* (truth and clarity) may be a conjugate pair connected in a

similar way as are position and velocity by the *Heisenberg* uncertainty principle. Thus, it seems not to be possible to make a given point clear without deviating from the truth, and this is comforting in view of some unavoidable oversimplifications.

Formation of Reactive Free Radicals in an Aqueous Environment

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2.1 General Remarks

Since DNA is a highly charged polyanion, it is always hydrated by water molecules [in the "dry" state (under moist air) it contains 12 water molecules per nucleotide subunit]. In a cellular environment, proteins (histones in eukaryotic cells) are always attached to DNA or are at least surrounded by proteins as in viruses. In order to attack DNA, radicals have to be sufficiently mobile in such a partially hydrophilic environment. For this reason, typical lipid radicals confined to the membranes will not be discussed here, although one must keep in mind that small fragments of free-radical nature may be able to escape the lipid environment and can, in principle, also react with DNA.

In the following, sources of reactive free radicals that may be produced in the cellular environment will be discussed. Artificial systems have also been widely used to study the free-radical chemistry of DNA, and the chemistry of such free-radical sources will also be mentioned here. Yet, it is beyond the scope of this book to deal exhaustively with all the various free-radical sources that have been used for the study of DNA free-radical chemistry. Some of them are straightforward such as the thermal or photolytic cleavage of compounds that are highly labile in the ground or excited state, and these aspects can be dealt with rather briefly. There are, however, areas such as radiolysis or sonolysis the principles of which are not common knowledge and must be dealt with in some more detail. In fact, a major part of our present knowledge of the free-radical chemistry of DNA is based on radiation-chemical studies, and hence this topic will be dealt with first. The formation of DNA radicals by the action of intercalating drugs such as bleomycin and neocarzinostatin will be discussed in Chapter 12.

2.2 Ionizing Radiation

To understanding the basis of radiation chemistry, it seems appropriate to recall some important aspects of the physics of energy absorption and the ensuing radiation chemistry, but for details the reader is referred to some textbooks, e.g., Henglein et al. (1969); Farhataziz and Rodgers (1987); von Sonntag (1987); Spinks and Woods (1990); Jonah and Rao (2001).

In the energy range below 1 MeV, the energy of high-energy photons, such as γ-rays and X-rays, is absorbed by the Compton and the photo effect. Above 1.01 MeV (twice the rest mass of the electron) pair formation, i.e., creation of an electron and a positron pair, starts to play a role. Both the Compton and the photo effect generate high-energy electrons which subsequently lose their energy as described by the Bethe equation. This equation relates the loss of energy (linear energy transfer, LET) of the charged high-energy particle to its mass, charge and energy. Multiply charged and heavy particles (e.g., α-particles) lose their energy more effectively (densely ionizing radiation) than singly charged light particles such as high-energy electrons (β-particles). As the energy of the electrons decreases, these sparsely ionizing particles also become more densely ionizing, i.e.,

they will cause further ionizations in the very neighborhood of their formation. Considering these mechanisms of energy absorption, it is understood why high-energy electrons (1–10 MeV) and γ-rays (60Co-γ-rays: 1.3 MeV, 137Cs-γ-rays: 0.5 MeV) have practically the same LET, and thus results obtained by γ-radiolysis can be compared with those from electron-beam irradiation.

Ionizing radiation is absorbed by the components of a given mixture approximately proportionally to the contributions by weight of the various components. Thus, when an aqueous DNA solution containing 500 mg dm^{-3} DNA is γ-irradiated, ~99.5% of the energy of the ionizing radiation is absorbed by the water and only ~0.5% by DNA. Under these conditions, the overwhelming contribution of free-radical damage to DNA will thus be caused by the free radicals generated by the radiolysis of water, and only a negligible part will arise from the absorption of the energy of the ionizing radiation by DNA itself (for the situation in a cellular environment see Chap. 12).

When the energy loss of high-energy electrons is absorbed by water, ~100 eV of energy is deposited per ionization event (on average), whereby a water radical cation and an electron is generated [reaction (1)]. The latter may still contain enough energy to cause further ionizations in the very near neighborhood. These areas, containing a number of ionization and occasionally also electronic-excitation events [reaction (2)], are called spurs. In the case of sparsely ionizing radiation these spurs do not overlap. In densely ionizing radiation, however, they indeed do forming cylinders of spurs called tracks, wherefrom some δ-rays (medium-energy electrons) may branch off (for the effects of high-LET radiation see, e.g., Swiatla-Wojcik and Buxton 1998).

$$H_2O \xrightarrow[\text{radation}]{\text{ionizing}} H_2O^{\bullet +} + e^- \quad (1)$$

$$H_2O \xrightarrow[\text{radation}]{\text{ionizing}} H_2O^* \quad (2)$$

The water radical cation, produced in reaction (1), is a very strong acid and immediately loses a proton to neighboring water molecules thereby forming $^{\bullet}OH$ [reaction (3)]. The electron becomes hydrated by water [reaction (4), for the scavenging of presolvated (Laenen et al. 2000) electrons see, e.g., Pimblott and LaVerne (1998); Pastina et al. (1999); Ballarini et al. (2000); for typical reactions of e_{aq}^-, see Chap. 4]. Electronically excited water can decompose into $^{\bullet}OH$ and $H^{\bullet}$ [reaction (5)]. As a consequence, three kinds of free radicals are formed side by side in the spurs, $^{\bullet}OH$, e_{aq}^-, and $H^{\bullet}$. To match the charge of the electrons, an equivalent amount of H^+ are also present.

$$H_2O^{\bullet +} \rightarrow {}^{\bullet}OH + H^+ \quad (3)$$

$$e^- + n\,H_2O \rightarrow e_{aq}^- \quad (4)$$

$$H_2O^* \rightarrow {}^{\bullet}OH + H^{\bullet} \quad (5)$$

Since a spur can contain more than one free-radical pair, there is always the possibility that they interact with one another [cf. the higher yields of e_{aq}^- and $^{\bullet}OH$

at very early times (Wolff et al. 1973; Jay-Gerin and Ferradini 2000); for the •OH yield reacting with plasmid DNA as a function of photon energy see Fulford et al. (1999); for a simulation of spur reactions see, e.g., Swiatla-Wojcik and Buxton (1995); Pimblott and LaVerne (1997)] in competition with an added solute (scavenger) (the complete set of rate constants pertinent to the radiolysis of water has been compiled by Buxton et al. 1988). Processes such as the reactions of •OH with the e_{aq}^- [reaction (6), $k = 3.0 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) or with H• [reaction (7), $k = 7.0 \times 10^9$ dm^3 mol^{-1} s^{-1}] will not lead to measurable final products. The self-termination of two •OH leads to H_2O_2 [reaction (8), $2k_8 = 1.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1}], while H_2 is formed in reactions (9) and (10) [$2k_9 = 1.55 \times 10^{10}$ dm^3 mol^{-1} s^{-1}; $2k_{10} = 1.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1}; for the kinetics of reaction (10) see, e.g., Schmidt and Bartels (1994)]. When the radicals diffuse out of the spur they may react with an added solute in competition (see below and Chaps. 3 and 4).

$$\bullet OH + e_{aq}^- \rightarrow OH^- \tag{6}$$

$$\bullet OH + H\bullet \rightarrow H_2O \tag{7}$$

$$2\,\bullet OH \rightarrow H_2O_2 \tag{8}$$

$$2\,H\bullet \rightarrow H_2 \tag{9}$$

$$2\,e_{aq}^- + 2\,H^+ \rightarrow H_2 \tag{10}$$

Often it is desirable to study the reaction of •OH without a contribution of e_{aq}^-. For this purpose, e_{aq}^- is usually converted into further •OH by saturating the solution with N_2O [reaction (11), $k = 9.1 \times 10^9$ dm^3 mol^{-1} s^{-1}; [N_2O] at saturation $= 2.2 \times 10^{-2}$ mol dm^{-3}].

$$e_{aq}^- + N_2O \rightarrow \bullet OH + N_2 + OH^- \tag{11}$$

H• and e_{aq}^- are in an acid/base equilibrium [pK_a(H•) = 9.1], and hence e_{aq}^- is converted into H• in acid solution [reaction (12), $k_{12} = 2.3 \times 10^{10}$ dm^3 mol^{-1} s^{-1}], while in basic solution H• is converted into e_{aq}^- [reaction (13), $k_{13} = 2.2 \times 10^7$ dm^3 mol^{-1} s^{-1}]. Saturation of the solution with O_2 ([O_2] = 2.5×10^{-3} mol dm^{-3}) converts H• and e_{aq}^- into $HO_2\bullet/O_2\bullet^-$ [pK_a(HO_2•) = 4.8; reactions (14) and (15), $k_{14} = 1.9 \times 10^{10}$ dm^3 mol^{-1} s^{-1} ; $k_{15} = 2.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1}]. Dioxygen does not react with •OH. Upon saturation the solution with a 4:1 mixture of N_2O and O_2, allows to convert e_{aq}^- practically fully into •OH [reaction (11)], and only the H• is scavenged by O_2.

$$e_{aq}^- + H^+ \rightarrow H\bullet \tag{12}$$

$$H\bullet + OH^- \rightarrow e_{aq}^- \tag{13}$$

$$e_{aq}^- + O_2 \rightarrow O_2\bullet^- \tag{14}$$

$$H\bullet + O_2 \rightarrow HO_2\bullet \tag{15}$$

Table 2.1. Radiation-chemical yields (G values; units: 10^{-7} mol J^{-1}) of water radicals, ions and molecular products at a scavenger capacity of ca. 2×10^6 s^{-1} under the conditions of sparsely ionizing radiation (^{60}Co-γ-rays, high-energy electrons) in the presence of different saturating gases (von Sonntag 1987)

Species	N_2	N_2O[a]	N_2O/O_2 (4:1)	O_2[a]
$^{\bullet}OH$	2.8	5.6	5.6	2.8
$H^{\bullet}$	0.6	0.6	–	–
e_{aq}^-	2.8	–	–	–
H^+	3.4	3.0	n.d.	n.d
OH^-	0.6	3.0	n.d.	n.d.
H_2O_2	0.7	0.9	n.d.	n.d.
H_2	0.5	0.5	0.5	0.5
$O_2^{\bullet -}/HO_2^{\bullet}$	–	–	0.6	3.3

[a] Approximate solubility at room temperature: $[N_2O] = 2.2 \times 10^{-2}$ mol dm^{-3}; $[O_2] \approx 1.35 \times 10^{-3}$ mol dm^{-3}; n.d. = not determined

The radiation-chemical yield is expressed by the G value. Originally, it has been given in the units of molecules formed (or destroyed) per 100 eV of absorbed energy. The eV is not an SI unit and hence usually no longer in use. These two units may be converted into one another taking into account that 1 molecule $(100\ eV)^{-1} = 1.036 \times 10^{-7}$ mol J^{-1}.

The number of $^{\bullet}OH$ that react in N_2O-saturated solutions with an added solute depends on its scavenging capacity (the product of the scavenging concentration times the rate constant of the scavenger with $^{\bullet}OH$). A formula that describes these dependencies approximately has been derived (Schuler et al. 1981), and a nomogram that follows this formula is given in von Sonntag (1987).

Most radiation-chemical studies have been carried out with scavenger capacities around 2×10^6 s^{-1}. In Table 2.1, the yields of $^{\bullet}OH$, e_{aq}^-, $^{\bullet}H$, and $O_2^{\bullet -}$ radicals are compiled for such a condition.

The rate constants of the reactions of $^{\bullet}OH$, e_{aq}^- and $^{\bullet}H$ with a large number of compounds have been determined by pulse radiolysis, and their values have been compiled (Buxton et al. 1988). Similarly, a large number of rate constants of the reactions of $HO_2^{\bullet}$ and $O_2^{\bullet -}$ are documented (Bielski et al. 1985). A more detailed picture of the radiation chemistry of water is given by Buxton (1987).

Although most experiments that use ionizing radiation have been carried out with γ-rays and high-energy particles, notably electrons, one has also to keep in mind that photons that exceed ~7 eV are capable of ionizing water. The photo-

ionization quantum yield increases with increasing photon energy and changes between 7.8 and 9.3 eV with an almost exponential dependence from $\Phi = 0.019$ to 0.22 (Bartels and Crowell 2000). Moreover, with high-intensity lasers ($>10^{11}$ W m^{-2}) non-trivial processes that lead to the photolysis/ionization of water by two-photon absorption via virtual states take place (Nikogosyan and Angelov 1981; Reuther et al. 1996; Görner and Nikogosyan 1997).

With substrates other than water, the photoionization may occur at photon energies <7 eV as a monophotonic process. With DNA in aqueous solution, photoionization is observed at 193 nm as a monophotonic process (Gurzadyan and Görner 1992; Candeias et al. 1992) (Chap. 11). With high-intensity lasers, biphotonic processes with quanta of lower energy (e.g., at $\lambda = 266$ nm) also give rise to photoionization. As a consequence, the ratio of the typical UV DNA damages, e.g., pyrimidine dimers, decrease with increasing laser intensity, while the free-radical-induced products such as DNA SSBs and DNA cross-links increase (Zavilgelsky et al. 1984).

In general, ionizing radiation produces in DNA and its model systems a large number of different radicals through the action of $^{\bullet}OH$, e_{aq}^{-} an $H^{\bullet}$. Yet in certain cases, one can also generate a given radical quite specifically by making use of the ready splitting of the C-Br bond by e_{aq}^{-}, for example in 6-bromo-5-hydroxydihydrothymine or in 5BrUra (Chap. 10).

2.3 Ultrasound

One of the phenomena observed with ultrasound is cavitation. Ultrasound created in a liquid is reflected at the liquid/gas interface and standing waves or fluctuating areas of sound nodes and antinodes develop. In the antinodes, the high-pressure and low-pressure phases change with frequency (frequencies used range typically between 20 and 1000 kHz). In the negative-pressure phase, small gas (e.g., air) bubbles may draw in some more gas and water vapor from the surrounding thereby growing to resonance size. In the compression phase (accelerated by the surface tension of the liquid), these gas bubbles heat-up adiabatically to several thousand degree (Flint and Suslick 1991; Tauber et al. 1999a; Rae et al. 2005). The contents of the gas bubble can decompose and give rise to free radicals and other reactive intermediates (Henglein 1987, 1993; Suslick 1990).

Besides small gas bubbles, other nucleation sites (e.g., at minute dust particles) may give rise to the cavitation phenomenon. Normally, the surface tension of water is too high to allow the formation of water vapor bubbles at the relatively small negative pressures created by the sonic field. However, at the surface of the dust particles the surface tension of water may be sufficiently low to create a water vapor bubble in the sonic field and thus start the cavitation process.

The oscillating gas bubbles are a continuous source of free-radicals as long as they remain in the antinode area of the sonic field, but one has also to envisage a catastrophic collapse that generates in addition to the free radicals a number of smaller bubbles. These serve as further nucleation sites for subsequent cavitation processes.

The temperature that is reached within the hot gas bubble depends on the heat capacity (C_p/C_v) of the saturating gas and its thermal conductivity. Diatomic gases (e.g., N_2 and O_2) have a C_p/C_v of 1.33 while the noble gases have only one of 1.0. A higher heat capacity of the gas lowers the temperature reached in the compression phase. Larger amounts of the vapor of the liquid drawn into the gas bubble have a cooling effect due to an increase in C_p/C_v. Among the noble gases, helium has the highest thermal conductivity. Thus, the final temperature of the collapsing bubble increases in the sequence He<O_2<Ar, and concomitantly increases the free-radical yield. Another very important factor is the temperature of the liquid. The surface tension of the liquid is a decisive force that determines the compression process, and since the surface tension vanishes at the boiling point, effective cavitation also ceases when the boiling point is approached either by increasing the temperature or reducing the pressure (Mark et al. 1998; von Sonntag et al. 1999).

In dilute aqueous solutions, the water vapor is decomposed into $^{\bullet}OH$ plus $H^{\bullet}$ [reaction (16)] or molecular hydrogen and an oxygen atom [reaction (17)].

$$H_2O \rightarrow {}^{\bullet}OH + H^{\bullet} \tag{16}$$

$$H_2O \rightarrow H_2 + O \tag{17}$$

The products of these reactions are thermally hot, and it has been suggested that even some $^{\bullet}OH$ are in their electronically excited state (Didenko et al. 1994). At these high temperatures, $H^{\bullet}$ carries enough energy to undergo the endothermic (63 kJ mol^{-1}; Benson 1965) reaction (18).

$$H^{\bullet} + H_2O \rightarrow H_2 + {}^{\bullet}OH \tag{18}$$

Since the concentration of the water vapor is most likely in excess over that of the free radicals formed at the short times while the bubble exists at very high temperatures, an excess of $^{\bullet}OH$ over $H^{\bullet}$ are formed (Anbar and Pecht 1964; Hart and Henglein 1985; Makino et al. 1983; Mark et al. 1998) [the view that essentially all water molecules in the cavitating bubble has also been put forward (Hart and Henglein 1986); for the frequency dependence of the $^{\bullet}OH$ yield see Mark et al. (1998)].

When the aqueous solution is saturated with O_2 or air, O_2 also can decompose into two O-atoms [reaction (19)]. There are two types of O-atoms, the triplet O-atom (ground state), and the singlet O-atom (excited state). The former reacts with O_2 giving rise to ozone which will not be stable in the hot bubble. The latter rapidly insert into water giving rise to a thermally hot H_2O_2 [reaction (20)]. In the gas phase, this decomposes into two $^{\bullet}OH$ [reaction (21)], while when the singlet O-atoms reach the bulk solution the thermally hot H_2O_2 rapidly loses its excess energy [reaction (22)], and only a small percentage gives rise to $^{\bullet}OH$ (for a discussion, see Reisz et al. 2003).

$$O_2 \rightarrow 2\,O \tag{19}$$

$$O + H_2O \rightarrow H_2O_2 \text{ (thermally hot)} \quad (20)$$

$$H_2O_2 \text{ (thermally hot) in the gas phase} \rightarrow 2\,^{\bullet}OH \quad (21)$$

$$H_2O_2 \text{ (thermally hot) in water} \rightarrow H_2O_2 \quad (22)$$

Upon the collapse of the bubble, the radicals (mainly $^{\bullet}OH$, see above) diffuse into the bulk solution, where they either recombine [reactions (23)–(25)] or react with a solute [reaction (26)].

$$2\,^{\bullet}OH \rightarrow H_2O_2 \quad (23)$$

$$H^{\bullet} + {}^{\bullet}OH \rightarrow H_2O \quad (24)$$

$$2\,H^{\bullet} \rightarrow H_2 \quad (25)$$

$$^{\bullet}OH + S \rightarrow S^{\bullet} \quad (26)$$

The concentration of radicals in the short liquid layer surrounding the bubble is very high (a value of approximately 10^{-2} mol dm^{-3} has been estimated; Gutierrez et al. 1991; von Sonntag et al. 1999), and scavenging of $^{\bullet}OH$ by a solute [reaction (26)] is very inefficient at low scavenger concentrations due to the competing reaction (23). Figure 2.1 exemplifies this point. Hydroxyl radicals react readily with iodide ions [reaction (27), $k = \times\ 10^9$ dm^3 mol^{-1} s^{-1}; (Buxton et al. 1988)], and as the iodide concentration is raised the yield of I_3^- [reactions (28) and (29)] increases while that of H_2O_2 decreases accordingly. However, molar concentrations of iodide are required to scavenge $^{\bullet}OH$ fully.

$$^{\bullet}OH + I^- \rightarrow OH^- + I^{\bullet} \quad (27)$$

$$I^{\bullet} + I^- \rightarrow I_2^{\bullet -} \quad (28)$$

$$2\,I_2^{\bullet -} \rightarrow I_3^- + I^- \quad (29)$$

Besides being degraded by $^{\bullet}OH$, volatile solutes are drawn into the gas bubble and decomposed there. As a consequence, low-molecular-weight solutes that are capable of protonation/deprotonation are degraded to a lesser extent when charged (Tauber et al. 2000). In addition, hydrophobic solutes may accumulate at the water/gas interface (Henglein and Kormann 1985; von Sonntag et al. 1999). This may cause two effects: they scavenge $^{\bullet}OH$ more readily than is accounted for by their scavenging capacity in the bulk, but they can also undergo excessive thermal degradation in this hot supercritical layer [experimental evidence for that transient state has not yet been obtained (Tauber et al. 1999b)].

In accordance with the above discussion, most of the damage encountered in DNA model systems (Mead et al. 1975; Kondo et al. 1988a,b, 1989, 1990) and in DNA (Fuciarelli et al. 1995) is due to $^{\bullet}OH$ reactions. Interestingly, ultrasound of

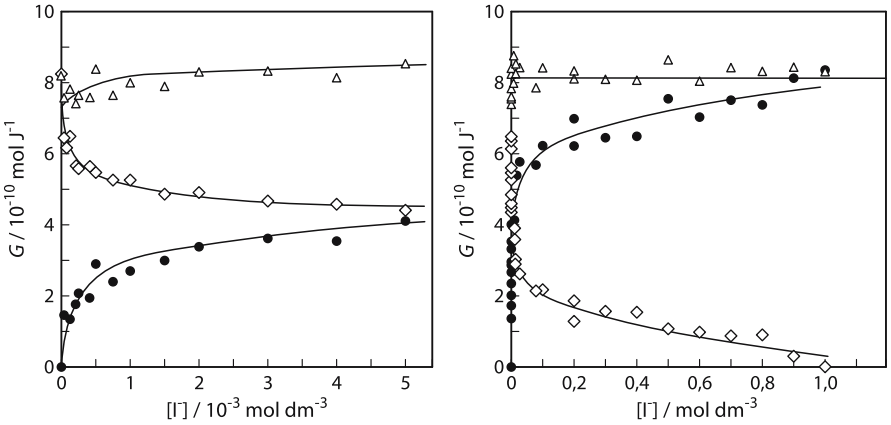


Fig. 2.1. Sonolysis of Ar-saturated water at 321 Hz and an intensity of 170 W kg^{-1}. Yields of I_2 (•), H_2O_2 (◊) and I_2 plus H_2O_2 (Δ) as a function of low concentrations of iodide (*left*) and as a function of high concentrations of iodide (*right*). Source: von Sonntag et al. (1999), with permission

the quality used in medicine seems not to have major damaging effects on the cellular DNA (Harder 1981).

2.4 Thermolysis and Photolysis

When a given bond is very weak, it may already be cleaved thermally at room temperature. Otherwise, elevated temperatures may be required for the reaction to proceed at a sufficiently high rate to be followed experimentally within a reasonable time. The necessary activation energy may also be provided by electronic excitation, but also bonds with relatively strong BDEs may be cleaved upon photolysis. Thus, photolysis is a very convenient way of creating free radicals and other reactive intermediates in aqueous solution. Some systems are discussed below. Since photochemical bond cleavage can be very selective, it allows us to generate very specifically a given radical in DNA model systems and to study mechanistic details of radicals that are also created by very reactive radicals such as •OH only in low yields. The great value of this technique is the possibility to incorporate such photolabile precursors even into dsODNs.

2.4.1 Thermolysis of Peroxides

The O-O bond in peroxides and hydroperoxides is often rather weak. Pernitrous acid already decomposes readily at room temperature (see below), and the decomposition of peroxodisulfate at 70 °C is used in technical processes, but H_2O_2

Table 2.2. O-O BDEs in some peroxides. (Brusa et al. 2000)

Peroxide	BDE /kJ mol^{-1}
H_2O_2	210 ± 2.5
Most organic hydroperoxides	201 ± 8.5
HC(O)OOH	201 ± 8.5
$CF_3C(O)OOH$	205 ± 8.5
$S_2O_8^{2-}$	120 ± 11
ONOOH	92 ± 8.5
ClOOCl	74.5 ± 8.5

is quite stable under such conditions. A compilation of some O-O BDEs is found in Table 2.2.

Among the reactive peroxides, peroxynitrous acid, ONOOH and its anion, peroxynitrite [pK_a(ONOOH) = 6.5–6.8 (Lögager and Sehested 1993; Goldstein and Czapski 1995; Kissner et al. 1997)] play an important role in cellular systems, and their reactions must be discussed here in some detail.

Nitric oxide ($NO^\bullet$) is generated enzymatically from glutamate by the nitric acid synthase and is responsible for a large number of diverse biological processes (for a review, see Nathan 1992; for spin trapping of the ensuing radicals, see Tsai et al. 2000). It was soon realized that an excess production of $NO^\bullet$ is toxic, and a part of its toxicity was attributed to its reaction with $O_2^{\bullet-}$, i.e., the formation of peroxynitrite [reactions (30) and (31)] (Beckman et al. 1990; for a review, see Murphy et al. 1998).

$$NO^\bullet + O_2^{\bullet-} \rightarrow ONOO^- \quad (30)$$

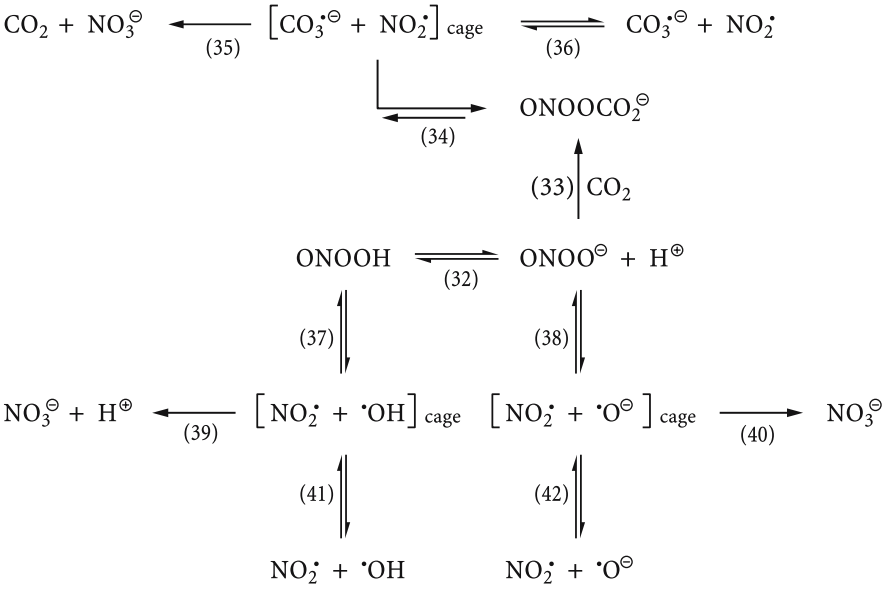
$$NO^\bullet + HO_2^\bullet \rightarrow ONOOH \quad (31)$$

For studying the chemistry of peroxynitrite in vitro, it may be prepared by reacting nitrous acid with H_2O_2 and subsequent rapid alkaninization (Hughes and Nicklin 1968) but also nitrite-free by ozonation of azide (Gleu and Roell 1929; Pryor et al. 1995) or from *S*-nitrosothiols with H_2O_2 (Coupe and Williams 1999). It also accumulates to substantial concentration upon the UV-photolysis of the nitrate ion in basic solution (Barat et al. 1969; Wagner et al. 1980; Mark et al. 1996) and is a product when air-saturated water is subjected to ultrasound (Mark et al. 2000). It may be detected by its absorption at 302 nm (Kissner et al. 1999).

The rate of reaction (30) is close to diffusion controlled [$k = 6.7 \times 10^9$ dm^3 mol^{-1} s^{-1} (Huie Padmaja 1993); 4.3×10^9 dm^3 mol^{-1} s^{-1} (Goldstein and Czapski 1995), 3.8×10^9 dm^3 mol^{-1} s^{-1} (Kobayashi et al. 1995); 1.9×10^9 dm^3 mol^{-1} s^{-1}

(Kissner et al. 1997)], and that of reaction (31) is not much slower [$k = 3.2 \times 10^9$ dm^3 mol^{-1} s^{-1} (Goldstein and Czapski 1995)]. The reaction of NO$^\bullet$ with $O_2^{\bullet-}$/$HO_2^\bullet$ is in competition with its autoxidation [following the rate law $-d[NO^\bullet]/dt = 4k[NO^\bullet]^2[O_2]$ [$4k = 8 \times 10^6$ dm^6 mol^{-2} s^{-1} (Awad and Stanbury 1993)]. In biological systems, the NO$^\bullet$ steady-state concentration is always low [for example, its intracellular steady-state concentration has been estimated at around 10^{-8} mol dm^{-3} (Liochev and Fridovich 1999)].

Peroxynitrous acid is a weak acid [equilibrium (32); for the pK_a see above]. Whereas the anion is rather stable, the acid rapidly decomposes quite rapidly [reaction (37)]; $k = 0.8$ s^{-1} at pH 7.5 (Kobayashi et al. 1995)] into $^\bullet NO_2$ and $^\bullet OH$ [for measurements of the volume of activation see Goldstein et al. (1999)] which either recombine in the cage yielding nitrate plus a proton [reaction (39)] or diffuse out of the cage giving rise to free $^\bullet NO_2$ and $^\bullet OH$ [reaction (41); Mark et al. (1996); Richeson et al. (1998); Coddington et al. (1999); 28 ± 4% yield (Gerasimov and Lymar 1999)].



For some time, there has been a discussion that $^\bullet OH$ is not the oxidizing species formed upon the decay of pernitrous acid (Koppenol et al. 1992; Koppenol 1998b, 1999). This conclusion was based on thermochemical grounds, because it had been inferred that in the reaction of $^\bullet OH$ with $^\bullet NO_2$ only ONOOH is formed, but there is now convincing evidence that this reaction leads to almost equal amounts of pernitrous and nitric acids (Merényi et al. 1999), supporting also thermochemical calculations concerning the feasibility of $^\bullet OH$-production (Merényi and Lind 1997). The situation got even more complex, when it was realized that CO_2 speeds up the decomposition of peroxynitrite (Keith and Powell 1969; Radi et al. 1993). There is now strong evidence for a peroxynitrite-CO_2 adduct [reaction (33)] (Denicola et al. 1996; Zhang et al. 1997; Lymar and Hurst

Table 2.3. Activation parameters for the decomposition of ONOOH and $ONOO^-$. (Merényi et al. 1999)

pH	$\Delta S^{\#}$/eu	$\Delta H^{\#}$/kcal mol^{-1}	E_a/kcal mol^{-1}	A/s^{-1}
4	13	21.2	21.8	1×10^{16}
14	–11	21.1	21.7	8×10^{10}

1998) [for corresponding adducts to aldehydes and ketones and their reactions see Meli et al. (1999); Merényi et al. (2002a,b)]. The peroxnitrite-CO_2 adduct is only very short-lived [reaction (34); (lifetime <100 ns (Goldstein et al. 2002)], and $CO_3^{\bullet -}$ and $NO_2^{\bullet}$ [reaction (36)] besides CO_2 and NO_3^- [reaction (35)] result (Goldstein and Czapski 1999; Meli et al. 1999; Merényi et al. 1999). The activation parameters for the decay of pernitrous acid and its anion are given in Table 2.3 (for a companison of the chemistries of peroxynitrites and peroxynitrates see Goldstein et al. 2005).

It is worth mentioning that the reduction potential of peroxynitrite is estimated at only 0.8 V, and many inorganic radicals, including $CO_3^{\bullet -}$, readily oxidize peroxynitrite (Goldstein et al. 1998) [reaction (43), $k = 9.6 \times 10^6$ dm^3 mol^{-1} s^{-1}]. The resulting $ONOO^{\bullet}$ radical has been suggested to decompose into $NO^{\bullet}$ (detected under oxidizing conditions) and O_2 [reaction (44)] (Mark et al. 1996).

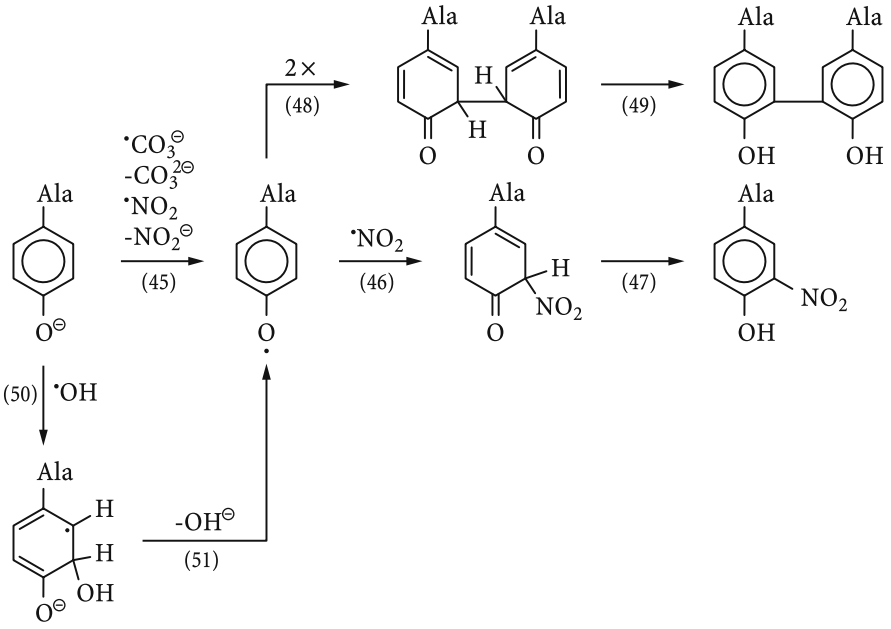
$$CO_3^{\bullet -} + ONOO^- \rightarrow CO_3^{2-} + ONOO^{\bullet} \quad (43)$$

$$ONOO^{\bullet} \rightarrow NO^{\bullet} + O_2 \quad (44)$$

In the presence of tyrosine, 3,3′-bistyrosyl and 3-nitrotyrosine are formed [reactions (45)-(51)], and the additional presence of CO_2 enhances their yield (Lymar et al. 1999). Tyrosyl radicals are specifically formed by the $CO_3^{\bullet -}$ and the $^{\bullet}NO_2$ radicals [reaction (45); the latter reacting more slowly: $k(^{\bullet}NO_2) = 2.9 \times 10^7$ dm^3 mol^{-1} s^{-1}; $k(CO_3^{\bullet -}) = 2.9 \times 10^8$ dm^3 mol^{-1} s^{-1}] (Neta et al. 1988), and since the for the nitration process a second $^{\bullet}NO_2$ radical is required [reaction (46)], the 3,3′-bistyrosyl to 3-nitrotyrosine ratio strongly depends on the reaction conditions (Lymar et al. 1999).

In analogy, the reaction of peroxynitrite with DNA results in a nitration of G to 8-NO_2-G and to its oxidation to 8-oxo-G (Yermilov et al. 1995, 1996). The presence of bicarbonate reduces peroxynitrite-mediated DNA strand breakage and the formation of TBA-reactive material from Thd (Yermilov et al. 1996).

It has been suggested that the main reason for the toxicity of $O_2^{\bullet -}$ is its conversion into peroxynitrite by $NO^{\bullet}$ and the ensuing deleterious effects of this compound as well as its CO_2-adduct (Koppenol 1998a, 1999). This suggestion has been challenged (Liochev and Fridovich 1999), and attention has been draw to the ready reaction of $O_2^{\bullet -}$ with [4Fe-4S] clusters [$k \approx 3 \times 10^6$ dm^3 mol^{-1} s^{-1} (Strohmeier Gort and Imlay 1998), which are estimated to reach concentrations



of 10^{-4} mol dm^{-3} in *E. coli* (Keyer and Imlay 1997)]. This will release iron ions that then may undergo Fenton reactions (see below). Unfortunately, our knowledge of the cellular steady-state concentrations of the various intermediates that we are concerned with here may not be sufficiently well established to reach final conclusions in this dispute.

In vivo, peroxynitrite may be intercepted by various cellular agents which will keep its steady-state low (Table 2.4). Not all these interceptors, however, react with peroxynitrite to non-reactive products. For example, carbon dioxide enhances tyrosine nitration and thiyl radical formation. Myeloperoxidase also enhances tyrosine nitration, and in the reactions with GSH and albumin thiyl radicals are formed (for details see Arteel et al. 1999).

This reduction of the lifetime of peroxynitrite by cellular components will, at least to a certain extent, protect DNA against the attack of this oxidant. The reactions of peroxynitrite with DNA and its model systems are discussed in Chapters 10 and 12.

2.4.2 Photolysis of Peroxides

Peroxides absorb in the UV range and are readily decomposed upon photoexcitation yielding two oxygen-centered radicals. Although the excited state is dissociative, the free-radical yield is never unity because of cage recombination reactions (Crowell et al. 2004). For example, the quantum yield of •OH-formation in the photolysis of H_2O_2 is only 1.0, i.e., the efficiency is only 0.5 (Legrini et al. 1993; Yu and Barker 2003). The peroxide chromophore is only weak [H_2O_2 or $S_2O_8^{2-}$: ε(254 nm)

Table 2.4. In vivo interception of peroxynitrite. (Arteel et al. 1999)

Reactant	In vivo concentration (mol dm^{-3})	Rate constant (dm^3 mol^{-1} s^{-1})	Rate of disappearance (s^{-1})
Spontaneous decay	–	–	0.4
Carbon dioxide	1×10^{-3}	3×10^4	30
Glutathione	1×10^{-2}	5.8×10^2	5.8
Ascorbate	1×10^{-2}	50	0.5
Myeloperoxidase	5×10^{-4}	4.8×10^6	2400
Glutathione peroxidase	2×10^{-6}	8×10^6	16
Hemoglobin	5×10^{-3}	2.5×10^4	125
Albumin	6×10^{-4}	5.6×10^3	3.4

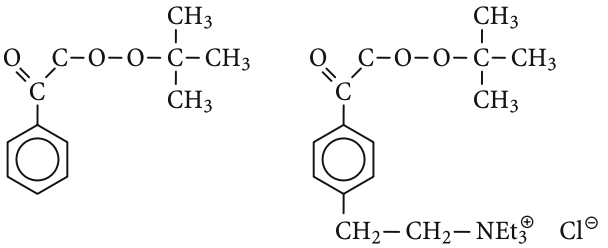
≈ 20 dm^3 mol^{-1} cm^{-1}, $\varepsilon(240\ nm) \approx 50$ dm^3 mol^{-1} cm^{-1} (Mark et al. 1990)], and in order to compete with other solutes present effectively for the incident photons, high concentrations of peroxides have to be added. This situation may change, when aromatic peroxides are used for the generation of the free radicals. In certain cases, the decomposition of peroxides can also be photosensitized using carbonyl compounds such as acetone (Behrens, private communication).

The oxyl radicals resulting from the decomposition of organic peroxides are energy-rich intermediates and undergo a number of rapid reactions (Chap. 7). Here, it is briefly recalled that the tertiary oxyl radicals undergo facile β-fragmentation [reaction (52)], and the primary and secondary oxyl radicals an equally fast water-assisted 1,2-H shift [reaction (53)].

$$R_3CO^{\bullet} \rightarrow {}^{\bullet}R + R_2C{=}O \tag{52}$$

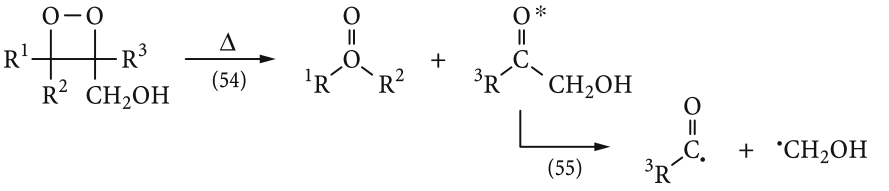
$$HR_2CO^{\bullet} \rightarrow {\bullet}C(OH)R_2 \tag{53}$$

Since reactions (52) and (53) are very fast, oxyl radicals are unlikely to react with substrates unless they are generated in their very close vicinity. For example, the perester shown below yields tertiary butoxyl radicals upon photolysis, but their efficiency to degrade dGuo is very low (Adam et al. 1998b). Most of the observed degradation of dGuo is due to reactions of the ensuing methylperoxyl radicals that are formed in the presence of O_2 (Adam et al. 2002), and in DNA only G is oxidized (Mahler et al. 2001). DNA strand breakage, caused by a H-abstraction from the sugar moiety (Chap. 12) is only induced by the photolysis of the perester which binds electrostatically to DNA (Adam et al. 1998b).



Even then, tertiary butoxyl radicals formed that close-by show only an SSB efficiency of about $1{:}10^5$.

1,2-Dioxetans undergo ready thermolysis into two ketones, one of them being formed in its excited triplet state which may undergo α-cleavage [reactions (54) and (55)]. The resulting radicals can cause DNA damage (Adam et al. 1998a).



2.4.3 Thermolysis of Azo Compounds

Azo compounds such as the water-soluble 2,2′-azobis(2-methylpropionamidine) dihydrochloride slowly decompose at elevated temperatures (Table 2.5) already at 40 °C, thereby yielding molecular nitrogen and two radicals [reaction (56)] (Niki 1990; Paul et al. 2000). These are first formed within the solvent cage, where about half of them recombines [reaction (57)]. The other half escapes the cage, and in the presence of O_2 these radicals are converted into the corresponding peroxyl radicals [reactions (58) and (59)].

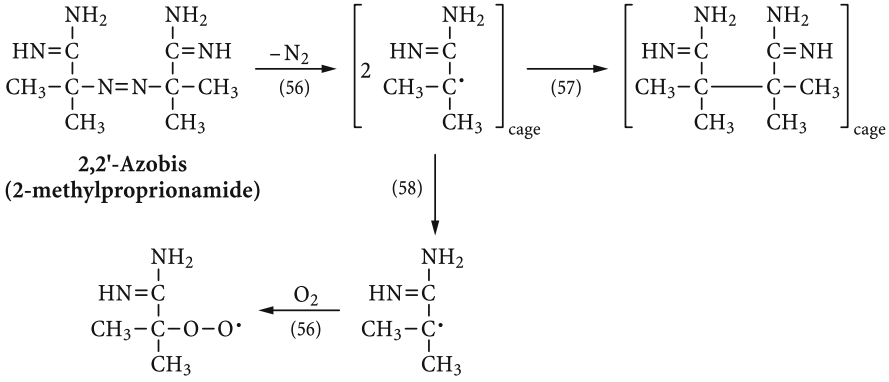


Table 2.5. Kinetic data for the decomposition of some azo radical sources at 37 °C in D_2O, pD ~ 7. (Paul et al. 2000)

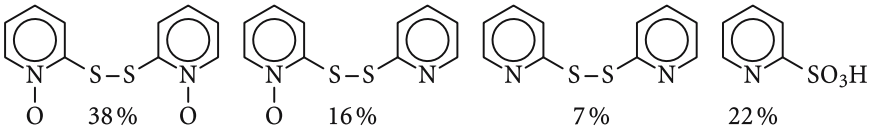
Azo compound	k/s^{-1}	$t_{½}/h$
2,2′-Azobis(amidinopropane) 2 HCl	1.3×10^{-6}	148
2,2′-Azobis(*N*,*N*′-dimethylisobutyramidine)	5.5×10^{-6}	37
Azobis[2-methyl-*N*-(2-hydroxyethyl)propionamide]	1.8×10^{-7}	1070
3,3′-Azobis(3-cyano-1-butanesulfonate 2 Na^+	3.5×10^{-7}	550
Di(4-carboxybenzyl)hyponitrite (SOST-1)	1.4×10^{-4}	1.3

This source of peroxyl radicals has been used to study the peroxyl radical reactions with nucleobases (Simandan et al. 1998) and thymidine (Martini and Termini 1997; Chap. 10). The slow H-abstraction reactions of peroxyl radicals prevents their reaction with DNA in dilute aqueous solution unless they are positively charged and thus bound to DNA by electrostatic forces (Paul et al. 2000). Otherwise, their competing bimolecular termination reactions are much faster (Chap. 8).

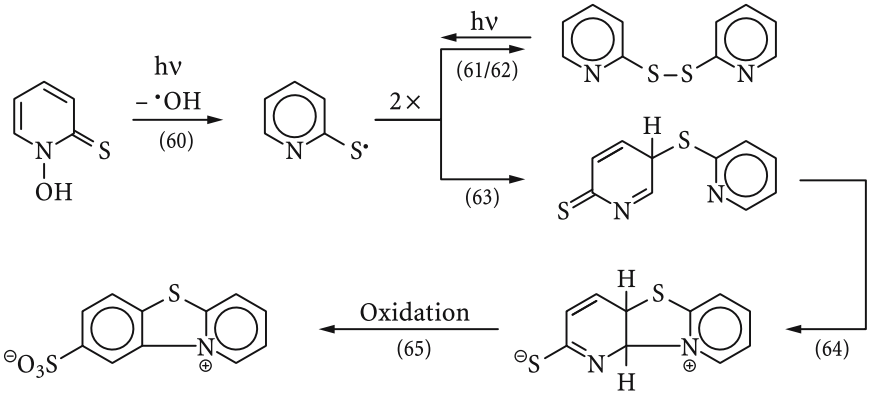
2.4.4 Photolysis of Mercaptopyridine-*N*-oxides

The absorption coefficient of H_2O_2 is rather poor (see above), and in the presence of strongly UV-absorbing compounds such as DNA or nucleobases, its photolysis cannot be used to generate •OH. Hence, the photolysis of 2- or 4-mercaptopyridine-*N*-oxide (Adam et al. 1998c) or *N*-hydroxy-4-(4-chlorophenyl)thiazole-2(3*H*)-thione (Adam et al. 2000b) at 350 nm as an •OH source [e.g., reaction (60)] has found its entrance in the study of DNA free-radical chemistry (Vieira and Telo 1997; Dias and Vieira 1997). The related *N*-alkoxypyrimidinethiones generate alkoxyl radicals whose reactions with DNA have also been investigated (Adam et al. 1998c).

Alternatively, *N*-hyroxypyridine-2-thione, a versatile antibacterial and antifungal agent commercially available as *Omadine*, has been used. As expected, in the presence of DNA its photolysis causes SSBs (via •OH), but after its photolytic consumption continuing photolysis at 350 nm caused the photooxidation of G in DNA and in dGuo (Adam et al. 1999). Apparently this effect is induced by one of its photolytic products that are largely the *N*,*N*′-dioxide, the mono-*N*-oxide, the disulfide and the sulfonic acid (Adam et al. 1999).



The formation of the latter two can be readily explained by a dimerization of the thiyl radicals [reaction (61)] formed in reaction (60) and a (slow) oxidation by O_2 which occurs in competition (no detailed mechanistic study is available at present that accounts for the other major products). The disulfide is not photostable, but slowly isomerizes to its head-to-tail isomer [reactions (62) and (63)], and in subsequent reactions is converted into the betaine [reactions (64) and (65)] which is the photocatalytic agent that causes the oxidation of the guanine moiety (Adam et al. 1999).



Thus, considerable care has to be taken not to misinterpret photobiological studies using this •OH-source. A further *caveat* has been expressed by Douki et al. (1999) who observed that product ratios are considerably different when ionizing radiation and *N*-hydroxypyridine-2-thione were used as •OH sources, and this has been explained by the H-donating property of the *N*-hydroxypyridine-2-thione (note the major dimers mentioned above).

2.4.5 Photolytic Generation of Specific Radicals

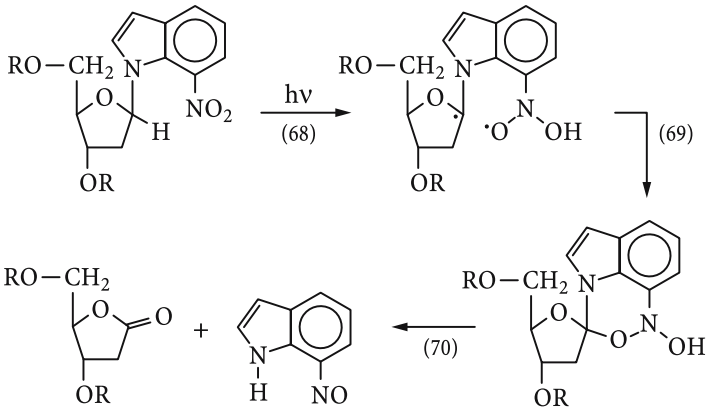
The generation of specific radicals/lesions within DNA is often achieved upon photolyzing adequately substituted derivatives such as *tert*-butyl ketone [reaction (66)] or phenylselenide derivatives [reaction (67)]. These and other reactions are discussed in Chapter 10.

$$\text{R-CO-C(CH}_3)_3 + h\nu \rightarrow \text{R}^\bullet + \text{CO} + {}^\bullet\text{C(CH}_3)_3 \qquad (66)$$

$$\text{R-Se-Ph} + h\nu \rightarrow \text{R}^\bullet + {}^\bullet\text{Se-Ph} \qquad (67)$$

Photolysis is also widely applied to generate the Ura-5-yl radical from 5-halouracils. In aqueous solution, the reaction is a homolytic splitting of the C-X (X = Br, I) bond (Chap. 10); in DNA, the reaction seems to be much more complex (Chap. 12).

Among other approaches (Chaps. 10 and 12), the 2-dRL lesion may be specifically generated by photolyzing the 7-nitroindol nucleoside (Kotera et al. 2000) [reactions (68)–(70)].



The *C*(1′) radical is only a short-lived intermediate in the reaction sequence.

2.4.6 Photosensitization

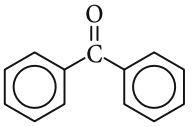
The term photosensitization is not well-defined. Mechanistically, it may comprise many different processes such as ET, H-abstraction, formation of singlet O_2 and reactive free radicals.

In many studies on the free-radical chemistry of DNA and its model systems, free-radical intermediates have been generated by photosensitization, notably using benzophenone (or its 3-carboxylate for better water solubility), 2-methyl-1,4-naphthoquinonone (menadione) or riboflavin. Among the anthraquinone derivatives, AQSO, AQS and 27AQS2 the latter shows selective cleavage of the single-stranded region of hairpin structures (Henderson et al. 1998).

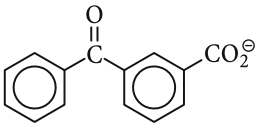
Upon photoexcitation, first the excited singlet state is reached which has only a very short lifetime (these compounds barely fluoresce at room temperature) and undergoes intersystem crossing into the triplet state. The triplet state has a much longer lifetime and hence can react with added substrates. Excited states can be good electron acceptors as well as electron donors. The above class mainly acts as electron acceptors [reaction (71)], while benzophenone ($Ph_2C{=}O$) and some quinones as long as their lowest excited state has n-π* character (cf. Schulte-Frohlinde and von Sonntag 1965) are also good H-abstractors [reaction (72)].

$$D + Ph_2C{=}O^* \rightarrow D^{\bullet+} + {}^{\bullet}C(Ph)_2{-}O^- \quad (71)$$

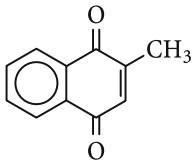
$$RH + Ph_2C{=}O^* \rightarrow R^{\bullet} + {}^{\bullet}C(Ph)_2{-}OH \quad (72)$$



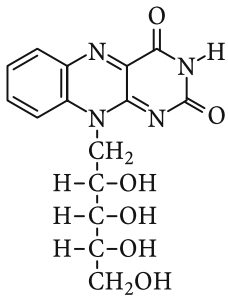
Benzophenone



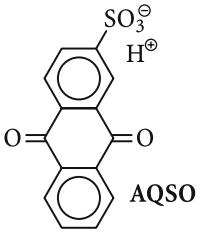
Benzophenone-3-carboxylate



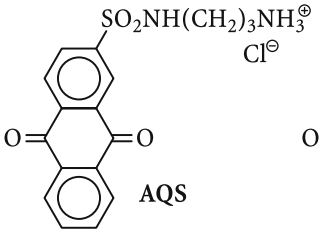
Menadione



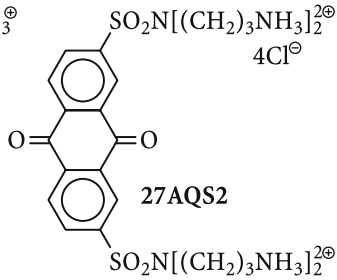
Riboflavin



AQSO



AQS



27AQS2

While in DNA, ET mainly occurs from G (preferentially from GG sites, cf. Ito et al. 1993; Saito et al. 1995, 1997; Saito and Takayama 1995; Sugiyama and Saito 1996; Ly et al. 1996; Gasper and Schuster 1997; Nakatani et al. 1998; Kino and Saito 1998), especially with intercalated quinones, non-selective DNA cleavage can occur with free H-abstracting quinones (Breslin and Schuster 1996). It may be mentioned here that 1,4-benzoquinone, and some other quinones, undergo a reaction with water (for details see von Sonntag et al. 2004), and these competitive reactions may have to be taken into account when using quinones as photosensitizer.

Triplet states are quenched by O_2 at near to diffusion-controlled rates. Quenching of high-energy triplets is dominantly product-less by a CT-type process. When the triplet energy of the sensitizer approaches that of the energy difference between that of O_2 triplet ground state and its first excited singlet state [$O_2(^1\Delta_g)$; $\Delta E = 96$ kJ mol^{-1}], energy transfer occurs [reaction (73)].

$$O_2 + S^* \rightarrow O_2(^1\Delta_g) + S \qquad (73)$$

This energy-rich O_2 has in water (H_2O) only a very short lifetime ($k = 2.5 \times 10^5$ s^{-1}; in D_2O it lives much longer, $k = 1.6 \times 10^4$ s^{-1}). Singlet O_2 shows often only a low reactivity, but with a series of compounds, including dGuo and its derivatives, it reacts quite rapidly (for a compilation of rate constants, see Wilkinson et al. 1995). Because of its prolonged lifetime in D_2O, the yields of $O_2(^1\Delta_g)$-reactions are much higher in this solvent than in water. In photosensitized reactions considered to occur by a free-radical pathway, these $O_2(^1\Delta_g)$-reactions may yield

unwanted by-products. To investigate $O_2(^1\Delta_g)$-reactions separately, one may produce $O_2(^1\Delta_g)$ separately by, e.g., using Rose Bengale and visible light (or H_2O_2 plus hypochloride; Muñoz et al. 2001). The triplet state of Rose Bengal lies too low to undergo ET or H-abstraction reactions from the kind of substrates that we are concerned with here.

The benzophenone ketyl radical reacts rapidly with O_2 yielding $O_2^{\bullet-}$ [reaction (74)].

$$^{\bullet}C(Ph)_2{-}O^- \; (^{\bullet}C(Ph)_2{-}OH) \; + \; O_2 \; \rightarrow \; Ph_2C{=}O \; + \; O_2^{\bullet-} \; (HO_2^{\bullet}) \qquad (74)$$

This type of reaction is also given by the menadione semiquinone radical (one-electron reduced menadione). However, the reduction potential of the riboflavin semiquinone [pK_a = 8.3 (Land and Swallow 1969); E_7 = -0.31 V (Anderson 1983a)] is so close to that of $O_2^{\bullet-}$ that ET to O_2 becomes very slow [k<3.8 × 10^4 dm^3 mol^{-1} s^{-1} for lumiflavin (Vaish and Tollin 1971), see also Faraggi et al. (1975)]. The flavin semiquinone radicals thus rather decay bimolecularly [their rate of decay depends on the charge, notable at the reaction site (Anderson 1983b), e.g. 2k($FH^{\bullet}$) = 1.2 × 10^9 dm^3 mol^{-1} s^{-1}, 2k($FAD^{\bullet-}$) = 1.2 × 10^9 dm^3 mol^{-1} s^{-1}, FAD = flavin-adenine dinucleotide]. They also readily react with $O_2^{\bullet-}$ [k = (2-7) × 10^8 dm^3 mol^{-1} s^{-1} (Anderson 1981, 1982)]. Thus, although the primary reaction may be the same for two photosensitizers, e.g., benzophenone and riboflavin, the products or their yields may be noticeably different whenever $O_2^{\bullet-}$ plays a major role in their formation. Whether this is one of the reasons for the differences observed with these photosensitizers in their reactions with some DNA model compounds (Delatour et al. 1999) has to await further investigation.

In contrast to the foregoing, porphyrins attached to DNA by, e.g., a positively charged polyethylenimine linker are thought to cause DNA strand breakage only by singlet O_2 reactions (Suenaga et al. 2000 and references cited therein).

Another widely investigated group of photosensitizers are ruthenium(II) and osmium(II) complexes which become strongly oxidizing in their excited state (for reviews see Kirsch-De Mesmaeker et al. 1996, 1998; Moucheron et al. 1997; Ortmans et al. 1998). As with other good one-electron oxidants, their primary site of attack is at G. Yet in contrast to the other systems discussed above, DNA-sensitizer cross-linking via G moiety and the ligands of the transition-metal ions (Jaquet et al. 1995) is often a major process (Kirsch-De Mesmaeker et al. 1998).

Photoexcitation of quinoxaline-carbohydrate hybrids cleave DNA selectively at GG sites (Toshima et al. 2002). Mechanistic details have not been explored, but the high GG specificity points to an ET in the excited state.

2.5 Transition-Metal Ions and Hydroperoxides

Cells contain low concentrations of transition-metal ions, notably iron and copper. For example, the DNA scaffolding protein is reported to contain copper (Lewis and Laemmli 1982). However, no intracellular free copper is detectable (Rae et al. 1999). The intracellular labile iron pool is reported to be around mi-

cromolar (Epsztejn et al. 1997). There is an ongoing debate as to whether only iron or also copper are involved in H_2O_2-induced DNA damage (Barbouti et al. 2001; Bar-Or and Winkler 2002; Galaris et al. 2002). The much higher steady-state level of damaged DNA bases in mitochondrial DNA as compared to nuclear DNA has been, at least in part, attributed to the relatively high levels of iron in mitochondria (Eaton and Qian 2002). It has been noted that supplementation of iron generates $^{\bullet}OH$ in vivo (Kadiiska et al. 1995). Other transition metal ions, e.g., nickel, cobalt, and chromium, may cause DNA damage (Nackerdien et al. 1991; Datta et al. 1993, 1994; Kasprzak 1996, 1997, 2002; Bal et al. 1997; Kasprzak et al. 1997). In aqueous solution, lipid hydroperoxides induce DNA strand breaks (besides base damage) that are considered to be mediated by adventitious DNA-bound transition metal ions (Yang and Schaich 1996). The nature of the hydroperoxide seems to play a mayor role including in the site-specificity (Inouye 1984; Ueda et al. 1985). Iron seems to play a major role in lipid autoxidation (Minotti and Aust 1989) and DNA damage occurs as a side reaction upon their elimination with the iron-containing peroxidases (Adam et al. 2000a). Thus, the chemistry of transition-metal ions with H_2O_2 and other hydroperoxides will be discussed in the following. It has been suggested, that in cellular systems "reductive stress" may reduce these transition-metal ions to their more reactive lower oxidation states and thus induce a situation commonly connected with "oxidative stress" (Ghyczy and Boros 2001). For all these considerations, it would be of importance to know the H_2O_2 concentration in cells. According to a review on the concentration of H_2O_2 in the human body (Halliwell et al. 2002), this seems be still an open question.

2.5.1 Iron

It has been observed by Fenton that Fe^{2+} and H_2O_2 yields a product with a much higher oxidation power that H_2O_2 itself [Fenton and Jackson (1899); for a historical review see Koppenol (1993); for general reviews see Goldstein et al. (1993); Sychev and Isak (1995); Wardman and Candeias (1996)]. This and related reactions are called Fenton or Fenton-type reactions. With amusement, we noticed that Schönbein (1857) made use of this reaction even earlier. In two exciting studies, Haber and Weiss have shown that this strong oxidizing property is due to the formation of a very short-lived entity which they have attributed to $^{\bullet}OH$ [reaction (75)] (Haber and Weiss 1932, 1934).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \qquad (75)$$

There is a continuing discussion, whether also (or exclusively) iron in a higher oxidation state [ferryl; Fe(IV)] is formed [reaction (76)] (Sawyer et al. 1996; Kremer 1999, 2000), but there is increasing evidence [for replies on the paper by Sawyer et al. (1996) see McFaul et al. (1998) and Walling (1998)] that the concept of a free $^{\bullet}OH$ being formed in this reaction can explain all the observed reactions and their kinetics, at least with aqua-Fe^{2+} in aqueous solution.

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow FeO^{2+} + H_2O \quad (76)$$

Reaction (76) has been believed to ruled out in a kinetic analysis of the Fenton reaction by EPR spin trapping (Mizuta et al. 1997) [a caveat is the observation that in water spin traps may be oxidized to the •OH-adduct via the spin trap radical cation by strong oxidants (Eberson and Persson 1997; von Sonntag et al. 2004)], but reaction (77), already suggested earlier (Rush and Koppenol 1987), was required to account for their data.

$$2\,Fe^{2+} + H_2O_2 \rightarrow 2\,Fe^{3+} + 2\,OH^- \quad (77)$$

If this reaction is of any importance a rather long-lived H_2O_2–Fe^{2+} complex or a Fe(IV) species must exist that can be further reduced by Fe^{2+}.

Ferryl species are well-documented and play a major role in P-450-type systems. In general, Fe(II)-containing enzymes try to avoid the formation of •OH in their reaction with H_2O_2. A similar situation seems to prevail in the case of Fe^{2+} complexed by DTPA (Rahhhal and Richter 1988), and one has to be keep in mind when discussing Fenton and Fenton-type reactions that complexation and possibly also the pH may shift the Fenton reaction from •OH to Fe(IV) as the reactive intermediate.

In aqueous solution and without artificial ligands, aqua-Fe(IV) is formed in the reaction of Fe^{2+} with ozone [reaction (78)] (Lögager et al. 1992; Jacobsen et al. 1997a,b), and under rather non-physiological conditions Fe(IV) and even higher oxidation states of iron have been generated and characterized by pulse radiolysis (Bielski 1991).

$$Fe^{2+} + O_3 \rightarrow FeO^{2+} + O_2 \quad (78)$$

Copper in its high oxidation state [Cu(III), formed in the reaction of Cu^{2+} with •OH] releases •OH in acid media (Meyerstein 1971; Cohen et al. 1990; Ulanski and von Sonntag 2000). Thus, it can also not be fully excluded also in the iron system a very short-lived Fe(IV) species is an intermediate on the way to •OH.

With aquo-Fe(II), the reaction has to be carried out in acidic media, because of the formation of precipitation in neutral solutions. The complexation of Fe(II) by ligands has a remarkable effect on the rate of reaction (Table 2.6).

A very common way to generate •OH in a Fenton reaction is the Udenfriend variation (Brodie et al. 1954), where in the original recipe Fe(II), EDTA, O_2 and ascorbate were used for aromatic hydroxylation. At the time, it had not been realized that Fe(II)EDTA and O_2 by themselves constitute a hydroxylating agent. It has only recently been shown that Fe(II) complexed to EDTA or NTA is readily oxidized by molecular O_2 (Seibig and van Eldik 1997; Zang and van Eldik 1990), and in a sequence of reactions •OH is generated (Yurkova et al. 1999). The formation of •OH by O_2 and Fe^{2+} has also been reported for complexes with polyphosphate (Biaglow and Kachur 1997) and nucleoside triphosphates (Biaglow et al. 1996; Kachur et al. 1997). The autoxidation of Fe^{2+} is also speeded up by phosphate buffer (Harris 1973), and from scavenging experiments it has been concluded that a Fe(IV) species rather than a free •OH is the reactive intermediate formed (Reinke

Table 2.3. Rate constants of Fenton-type reactions. For further details see Goldstein et al. (1993) and Koppenol (1994)

Couple	E°/V	pH	k/dm^3 mol^{-1} s^{-1}
$Fe^{II/III}$	0.77	1	65
$Fe(EDTA)^{II/III}$	0.12	7	7×10^3
$Fe(pyrophosphate)^{II/III}$		7.2	1×10^5
$Fe(cyt.c)^{II/III}$	0.27	7	2.5
$Cu^{I/II}$	0.16	1	4×10^3
$Cu(OP)^{I/II}$	0.17	7	2×10^3
$Cr^{II/III}$	−0.41	0	7×10^4
$Ti^{III/IV}$	0.1	0	540
$V^{II/III}$	−0.26	1	8.6
VO^{2+}/VO^{3+}	1.0	0	~1
$Ru(NH_3)^{II/III}$	0.067	7	~1.5
$Co([14]aneN_4)^{II/III}$	0.44	1	4×10^3

et al. 1994). Under such conditions, dGuo is oxidized to 8-oxo-dGuo (Svoboda and Harms-Ringdahl 2002). Complexation of Fe^{2+} to the phosphate groups of DNA is even more effective as the autoxidation of Fe^{2+} is concerned (for the role of transition metal ions in the catalysis of the autoxidation of biomolecules, see Miller et al. 1990). EDTA and DPTA are often used to complex iron and prevent its interference in autoxidation reactions, and there is an ongoing discussion as to the molecular basis of the reasons why these ligands may act under certain conditions as an antioxidant or a pro-oxidant (Engelmann et al. 2003).

Due to the ready reaction of complexed Fe(II) with O_2 and the generation of $^{\bullet}OH$ in the course of this reaction, the sequence of the addition of the reagents will be of a major importance especially when H_2O_2 is used to induce a Fenton reaction. In fact, when H_2O_2 is added last and somewhat late, there may be no Fe(II) left to induce the Fenton reaction proper. In many studies this potential artifact has not been realized (e.g., Asaumi et al. 1996 as an example), and conclusion are potentially fraught with considerable errors.

When such reactions are allowed to proceed in the presence of 2′-deoxynucleosides, different product ratios than generated by ionizing radiation are observed (Murata-Kamiya et al. 1998). This is likely to be due to the presence of both Fe(II) and Fe(III) which may modify the $^{\bullet}OH$-induced reactions (Theruvathu et al. 2001b). Such phenomena may be quite a general feature whenever Fe(II) [for rate constants see Khaikin et al. (1996)] and/or another reductant (e.g., ascorbate) is involved.

These days, quite common procedures use for the Udenfriend reaction H_2O_2 as an $^{\bullet}OH$ source and catalytic amounts of Fe(III) which are reduced by the ascorbate, for example, 5×10^{-3} mol dm^{-3} substrate, 5×10^{-6} mol dm^{-3} Fe(III), 15×10^{-6} mol dm^{-3} EDTA, 50×10^{-6} mol dm^{-3} H_2O_2 and 50×10^{-6} mol dm^{-3} ascorbate (Halliwell and Kaur 1997). Here, one has to take into account that both Fe(II)EDTA and ascorbate (cf. Huie et al. 1987; Neta et al. 1989) reduce intermediate peroxyl radicals [reactions (79)–(82); cf. Yurkova et al. 1999; for the one-electron reduction of hydroperoxides see Phulkar et al. 1990].

$$^{\bullet}OH + RH \rightarrow R^{\bullet} + H_2O \quad (79)$$

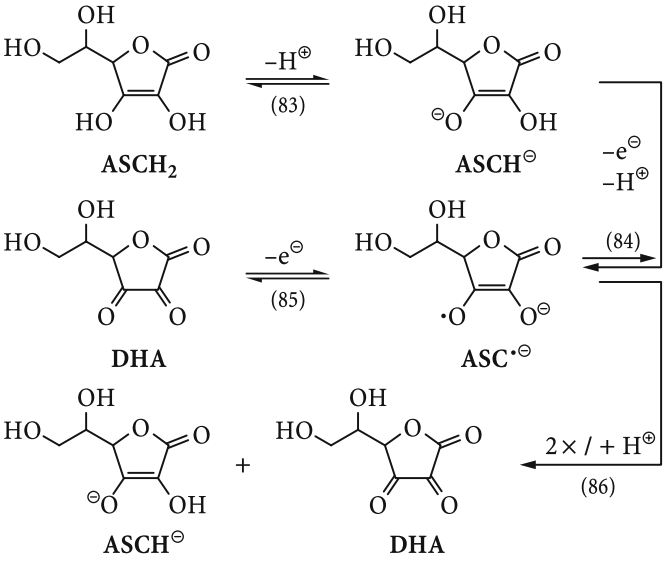
$$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet} \quad (80)$$

$$RO_2^{\bullet} + \text{reductant} \rightarrow RO_2H \quad (81)$$

$$RO_2H + Fe^{2+} \rightarrow RO^{\bullet} + OH^- + Fe^{3+} \quad (82)$$

Because of this apparent importance of ascorbate not only in artificial systems (for the $^{\bullet}OH$-production by multivitamin tablets, see Maskos and Koppenol 1991) but also in the living cell where a concentration of ca. 10^{-2} mol dm^{-3} is typically maintained, it seems adequate to digress for a short moment on its chemistry.

Ascorbic acid [ε(244nm) = 10,800 dm^3 mol^{-1} cm^{-1}] is an acid (pK_a = 4.1) [equilibrium (83)]. Thus at around pH 7 the monoanion [ε(265nm) = 14,500 dm^3 mol^{-1} cm^{-1}] predominates.



The ascorbate ion is a reasonably strong reductant [E^7($ASC^{\bullet -}$, H^+/$ASCH^-$) = 0.282 V; equilibrium (84)]. In the presence of its oxidized form, dehydroascorbic acid [DHA, ε(300nm) = 720 dm^3 mol^{-1} cm^{-1}], an equilibrium concentration of

the ascorbate radical [ε(360nm) = 3300 $dm^3\ mol^{-1}\ cm^{-1}$] is established according to Eq. (87), where pK_1 is the first ionization constant of ascorbic acid and $[ASCH_2]_{total}$ is the analytical concentration of ascorbic acid, i.e. $[ASCH_2]_{total} = [ASCH_2] + [ASCH^-] + [ASC^{2-}]$.

$$K = \frac{[ASC^{\bullet -}]^2[H^+] + \{1+[H^+]/10^{-pK_{85}}\}}{[DHA][ASCH_2]_{total}} = 2.0 \times 10^{-15}\ dm^{-6}\ mol^2 \qquad (87)$$

The bimolecular decay of the ascorbate radical is much more complex than shown in the overall reaction (86). In fact, it is in equilibrium with a dimer [equilibrium (88), $K \approx 10^3\ dm^3\ mol^{-1}$, $k_{reverse} \approx 10^5\ s^{-1}$] which either may react with a proton [reaction (89), $k \approx 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$] or with water [reaction (90), $k \approx 40\ s^{-1}$] (Bielski et al. 1981).

$$2ASC^{\bullet -} \rightleftharpoons (ASC^{\bullet -})_2 \qquad (88)$$

$$(ASC^{\bullet -})_2 + H^+ \rightarrow ASCH^- + DHA \qquad (89)$$

$$(ASC^{\bullet -})_2 + H_2O \rightarrow ASCH^- + DHA + OH^- \qquad (90)$$

Transition-metal-ion-free solutions of ascorbate autoxidize (i.e., react with O_2) only slowly (e.g., carefully demetalized with a chelating resin such as Chelex 100 1.25×10^{-4} mol dm^{-3} solutions lose only 0.05% ascorbate/15min (Buettner 1988; for a review giving valuable information how to deal with ascorbate solutions, see Buettner and Jurkiewicz 1995). The stability of ascorbate solutions is dramatically reduced in the presence of EDTA which apparently catalyzes the degradation by chelating adventitious iron ions (Buettner and Jurkiewicz 1995).

When using the Udenfriend reaction for producing $^{\bullet}OH$, one has to keep in mind that $^{\bullet}OH$ reacts very fast with ascorbate ($k = 7.9 \times 10^9\ dm^3\ mol^{-1}\ s^{-1}$; Redpath and Willson 1973) whereby not only the $ASC^{\bullet -}$ radical but also adduct radicals are formed (Fessenden and Verma 1978; see also Schuler et al. 1974; Cabelli and Bielski 1983; Cabelli et al. 1984).

Thiols, e.g., DTT, are also capable of reducing Fe^{3+} under certain conditions and thus induce autoxidation processes (Netto and Stadtman 1996). The kinetics are very complex.

In the context to the question of Fe^{2+}/H_2O_2-mediated DNA damage the question has to be raised to what extent iron is readily available in the cell or can be released under certain stress conditions. In cells, iron homeostasis is highly regulated, and bacteria, yeast and mammalian cells follow different strategies [for a review see Meneghini (1997)]. Only small amounts of so-called chelatable iron (i.e., can be detected/extracted with iron chelators such as desferrioxamine or 1,10-phenanthroline) are freely available in the cell. Most of the iron is tightly bound to ferritin (for the importance of ferritin as an iron source for oxidative damage, see Reif 1992), heme groups and iron-sulfur clusters (cf. Fig. 2.2). The release of iron under the conditions of oxidative stress has bee reviewed (Comporti et al. 2002).

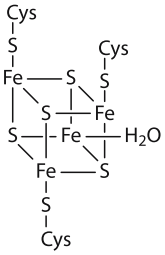
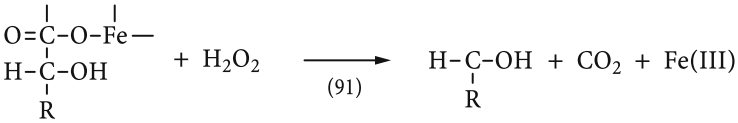


Fig. 2.2. Cubane of iron-sulfur cluster (4Fe-4S), prostetic group of cytosolic aconitase

5-Aminolevulinic acid, an element in the biosynthesis of heme proteins, may be overexpressed under certain pathological conditions and its accumulation has been correlated with some hepatitic cancers. It is in equilibrium with its enol form and can complex transition-metal ions. In the presence of O_2, this may lead to the formation of $^{\bullet}OH$ and hence in the presence of DNA to DNA damage which is enhanced in the presence of ferritin (Douki et al. 1998; di Mascio et al. 2000). Superoxide radicals have been assumed to be intermediates in these reactions. Mechanistically, the formation of $O_2^{\bullet -}$ is certainly very complex, because even in the case of the Fe(II)-EDTA-complex the reduction potential is not low enough to reduce O_2 by simple one-electron donation.

The Fenton reaction may also be used site-specifically, *e.g.* for the sequence-specific cleavage of DNA with the help of benzopyridoindole-EDTA intercalator forming triple-helical structures (Marchand et al. 2000).

There are some reactions that use Fe(III) and H_2O_2 such as the Ruff degradation of aldonic acids [reaction (91); Moody 1964]. Here, Fe^{3+} only acts as a catalyst and free radicals seem not to be involved in this reaction.



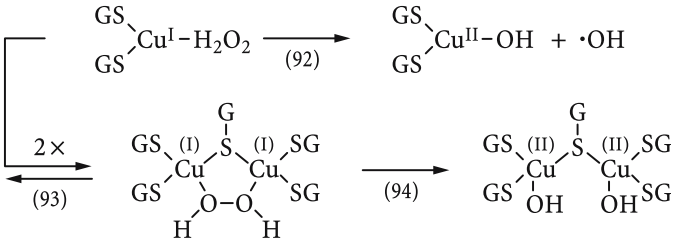
In this context, the report that in the Fenton reaction the $^{\bullet}OH$ yield (as determined by spin trapping) is considerably increased in the presence of α-hydroxy acids (Ali and Konishi 1997) may find an explanation if the α-hydroxy acid/Fe(III)/H_2O_2 complex oxidizes the spin trap thereby mimicking an increased yield of $^{\bullet}OH$.

The Gif chemistry (Perkins 1996; Barton 1996, 1998) that uses complexed Fe(III) plus H_2O_2 is carried out in organic solvents and thus is of no relevance here, although some of the underlying chemistry may be of some relevance in the action of BLM.

2.5.2 Copper

There are a number of transition-metal ions in low oxidation states such as Cu(I) which readily react with H_2O_2. In the case of Cu(I), the resulting intermediate has also a very strong oxidative power, e.g., is capable of abstracting an H atom from MeOH. At high MeOH concentration the chain length of this reaction depends on the MeOH concentration, and it has thus been concluded that an oxidant must be present which has not the properties of free •OH (Johnson et al. 1985, 1988). Evidence has been given that the intermediate, $(H_2O)_mCu^+$-O_2H^- can decompose in acid solution into Cu^{2+} and •OH, but at sufficiently high H-donor concentration also react with the latter (Masarwa et al. 1988).

In the reduction of Cu^{2+} to Cu^+, Cl^- may play an important role, because they considerably stabilize the Cu^+ species (Gilbert et al. 1997). GSH is the major cellular free thiol (close to 10^{-2} mol dm^{-3}). It readily reduces Cu(II) to Cu(I)-GSH complexes. These react with hydroperoxides (Gilbert and Silvester 1997; Gilbert et al. 1999). At low concentrations where these complexes are monomeric, the reaction with H_2O_2 gives rise to •OH [reaction (92)], but higher concentrations the monomeric complexes aggregate [reaction (93)] and this aggregate undergoes a two-electron oxidation [reaction (94)]. In contrast, *tert*-butylhydroperoxide undergoes one-electron oxidation also with the oligomeric aggregates. This has been rationalized by pointing out that at pH 7 the one- and two-electron potentials are 0.46 and 1.32 V for H_2O_2 and 1.9 and 1.7 for *tert*-butylhydroperoxide, respectively. Thus a two-electron step is thermodynamically preferred for H_2O_2, whereas the reverse is true for *tert*-butylhydroperoxide.

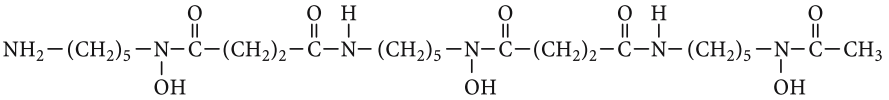


The above reactions may play a role in copper-mediated DNA damage, and the effect of the *trans*-resveratrol, a naturally occurring phenolic antioxidant, has been studied in this context (Burkitt and Duncan 2000).

Albumine-Cu^{2+} complexes are similarly reduced by thiols and then react with H_2O_2 (Ozawa et al. 1993). The •OH radical thus formed has been detected by spin trapping.

DNA strand breakage is caused by EDTA-Cu^{2+} in the presence of a thiol (Mukherjee and Chatterjee 1995). In this study it has, however, also been reported that Cu^{2+} (in the presence of H_2O_2 and Cl^-) nick DNA in the absence of a deliberately added reductant. The latter observation has been corroborated by Yamamoto and Kawanishi (1989), and it has been suggested that H_2O_2 can serve as a reductant under these conditions. The pattern of DNA fragments in

the presence and absence of a reductant is markedly different (John and Douglas 1996) pointing to different mechanisms. While the Fe^{2+} desferal complex, a highly effective chelation drug in iron overload diseases, does not undergo Fenton-type reactions, its Cu, Co and Ni complexes are capable of cleaving DNA (Joshi and Ganesh 1994).



Desferal

The DNase activity of *o*-phenanthroline-Cu^{2+} complex bound to DNA in the presence of H_2O_2 is also triggered by thiols (Chap. 12).

2.5.3 Manganese

Manganese(II) is normally not readily autoxidized, but when it is complexed by, e.g., nitrilobis(methylenephosphonic acid) ready autoxidation by O_2 occurs (Nowack and Stone 2000). Whether under such conditions •OH are set free as in the autoxidation of EDTA-complexed Fe(II) (Yurkova et al. 1999), is not yet know.

2.6 Ozone

Ozone is widely used for the disinfection of drinking water (Ellis 1991), and especially in the case of viruses there is a considerable likelihood that the nucleic acids are the main target (Roy et al. 1981). Ozone has been reported to be weakly mutagenic (Dubeau and Chung 1982; Dillon et al. 1992; Rodrigues et al. 1996), and DNA damage is reported for plants and human tissues (Fetner 1962; Huber et al. 1971; Floyd et al. 1989). In its reaction with DNA, a part of the DNA damage is due to •OH (van der Zee et al. 1987). This may potentially be due to its reaction with A, for which there is increasing evidence that •OH are generated at one stage of the reaction sequence (Ishizaki et al. 1984; Theruvathu et al. 2001a). Thus, ozone may be included into the reagents that cause DNA damage by a free-radical pathway, although most of the ozone reactions that have been studied so far are non-radical in nature.

Ozone is a strongly electrophilic reagent (for a compilation of rate constants, see Neta et al. 1988), and in its reaction with aromatic compounds its rate of reaction strongly depends on the electron-donating/withdrawing power of the substituent (ρ = -3.1; Hoigné and Bader 1983). A similarly dramatic substituent effect is observed with olefins, where the reaction rate constant varies by as much as eight orders of magnitude on going from tetramethyl- to tetrachloroethene [Dowideit and von Sonntag 1998;for the rate constants of ozone with the

nucleobases, see Theruvathu et al. 2001a; for the complex (non-radical) ozone chemistry of Thy and Thd see Flyunt et al. 2002].

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3.1 General Properties

The various ways of forming •OH were discussed in Chapter 2. It is a very reactive, electrophilic (ρ = -0.41; Anbar et al. 1966a) radical, and with most substrates it reacts at close to diffusion-controlled rates (for a compilation of rate constants, see Buxton et al. 1988). It undergoes mainly three types of reactions: (1) addition to C-C and C-N double bonds, (2) H-abstraction and (3) ET. Addition and H-abstraction reactions will be discussed below in some detail, because they are relevant for an •OH-attack at the nucleobases and at the sugar moiety in DNA.

Although it is of little importance at the pH values that prevail in biological systems, it must be mentioned that at high pH •OH deprotonates [equilibrium (1), pK_a(•OH) = 11.8 (Weeks and Rabani 1966), 11.54 (Poskrebyshev et al. 2002); for its temperature dependence see Elliot and McCracken (1989); for UV spectra see Czapski and Bielski (1993) and Poskrebyshev et al. (2002)].

$$^{\bullet}OH + OH^- \rightleftharpoons O^{\bullet -} + H_2O \quad (1)$$

The $O^{\bullet -}$ radical may also be generated in neutral solution (e.g., $e_{aq}^- + N_2O \rightarrow O^{\bullet -} + N_2$; $NO_3^- + h\nu \rightarrow {}^{\bullet}NO_2 + O^{\bullet -}$), but it is so rapidly protonated by water ($k_{-1} \approx 10^8$ s^{-1}) that its reaction with a given substrate can be neglected (cf. Mark et al. 1996), and only the typical •OH reactions are observed. Thus $O^{\bullet -}$ reactions only occur to a significant extent at pH >12.

The (nucleophilic) $O^{\bullet -}$ radical still undergoes H-abstraction at a high rate (for the energetics, see Henglein 1980), but its ability to add to double bonds is strongly reduced (for a compilation of rate constants, see Buxton et al. 1988). For example, in nucleosides the preference of attack at the base is shifted at high pH towards a pronounced H-abstraction from the sugar moiety (Scholes et al. 1992). An important difference between the (electrophilic) •OH and its conjugate base, the (nucleophilic) $O^{\bullet -}$, is their reactivity towards O_2. While O_2 does not react with •OH, it readily adds to the electron-rich $O^{\bullet -}$, yielding the ozonide radical anion [equilibrium (3); pK = 6.26; for details, see Elliot and McCracken 1989].

$$^{\bullet}OH + O_2 \rightarrow \text{no reaction} \quad (2)$$

$$O^{\bullet -} + O_2 \rightleftharpoons O_3^{\bullet -} \quad (3)$$

3.2 Addition to Double Bonds

The electrophilic •OH reacts readily with C-C and C-N double bonds (in purines) but not with C-O double bonds which are electron-deficient at carbon, the position where •OH would prefer to add. Although •OH reacts with C-C double bonds at close to diffusion-controlled rates, it is highly regioselective largely due to its

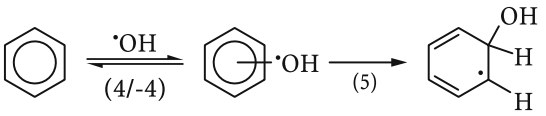
Table 3.1. Hydroxyl-radical-induced hydroxylation of some benzene derivatives in the presence of $Fe(CN)_6^{3-}$ serving as an oxidant of the •OH adducts. Yields (in %) relative to the •OH yield

Substrate	ortho	meta	para	Other products	Reference
Anisole	48	8	40	Phenol (6)	Steenken and Raghavan (1979)
Phenol[a]	48	3	36		Raghavan and Steenken (1980)
Chlorobenzene	45	22	31	HCl (0.5)	Merga et al. (1996)
Benzoic acid	28	38	20	Phenol (7)	Klein et al. (1975)
4-Methylphenol			~12		Schuler et al. (2002)

[a]Using 2,5-dimethylbenzoquinone as oxidant

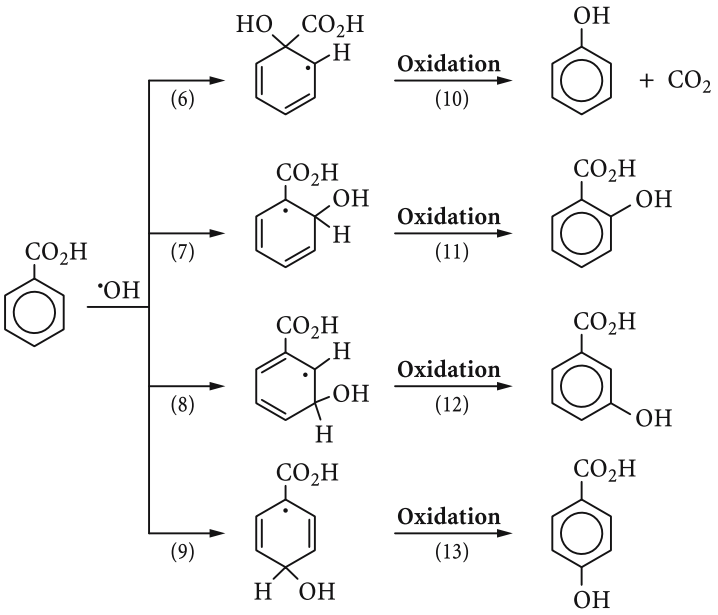
electrophilic nature. For example, the *C*(5)–*C*(6) double bond in the pyrimidines is preferentially attacked at the electron-richer *C*(5) (Chap. 10).

It has been suggested that •OH fixation at a given carbon may be preceded by a short-lived π-complex (Cercek and Ebert 1968; Volkert and Schulte-Frohlinde 1968). There is now experimental evidence from high-temperature pulse radiolysis studies that this view may be correct (Ashton et al. 1995). While the formation of the π-complex is a reversible reaction [equilibrium (4)], once the σ-complex is formed [reaction (5)] the OH group remains tightly bound. The high regioselectivity of •OH addition reactions may, in fact, occur at the transition from the π- to the σ-complex.

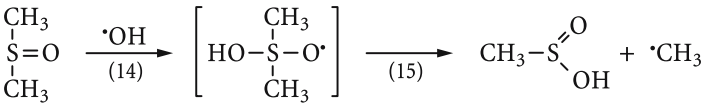


π-complex σ-complex

In benzene derivatives, electron-donating substituents direct into the *ortho*- and *para*-positions, while in the case of the electron-withdrawing substituents considerable *meta*-addition is observed (Table 3.1); otherwise a more equal distribution is established [reactions (6)–(9) and Table 3.1]. In agreement with the pronounced regioselectivity, *ipso*-addition at a bulky substituent such as the chlorine substituent in chlorobenzene is disfavored. Evidence for this is the low HCl yield in the case of chlorobenzene, the low yield of *para* adduct in 4-methylphenol (Table 3.1), or the decarboxylation in the case of benzoic acid [reactions (6) and (10)].



With sulfoxides, •OH reacts mainly by addition to the S-O double bond [DMSO: reaction (14); 92%; $k = 7 \times 10^9$ dm^3 mol^{-1} s^{-1}]. The resulting adduct has not been detected, because it decomposes very rapidly by β-fragmentation [reaction (15); Dixon et al. 1964; Norman and Gilbert 1967; Veltwisch et al. 1980].

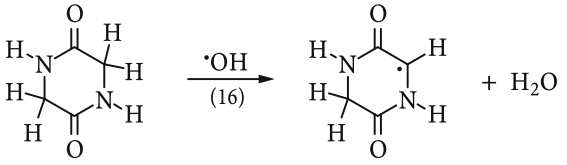


3.3 H-Abstraction

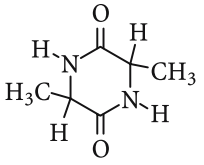
The HO-H bond dissociation energy (BDE) is 499 kJ mol^{-1}, while the C-H bonds in saturated hydrocarbons are much weaker (BDE = 376–410 kJ mol^{-1}; Berkowitz et al. 1994; for a compilation, see Chap. 6). Thus, there is a considerable driving force for H-abstraction reactions by •OH. On the other hand, vinylic hydrogens are relatively tightly bound, and an addition to the C-C double bond is always favored over an H-abstraction of vinylic or aromatic hydrogens. Hence, in the case of ethene, no vinylic radicals are formed (Söylemez and von Sonntag 1980), and with benzene and its derivatives the formation of phenyl-type radicals has never been conclusively established.

Despite the considerable driving force for the H-abstraction reaction, there is some remarkable selectivity. Primary hydrogens (-CH_3) are less likely abstracted than secondary (-CH_2–) and tertiary (-CH-) ones (Asmus et al. 1973). In addition, neighboring substituents that can stabilize the resulting radical by elec-

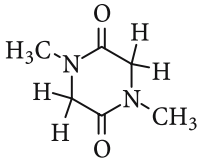
tron donation [such as -OR or -NR_2] favor abstraction of a given hydrogen atom. On the other hand, neighboring electron-withdrawing groups such as carbonyl functions decrease the rate of H-abstraction by the electrophilic •OH. Thus, its rate with acetic acid is rather low ($k \approx 10^7$ dm^3 mol^{-1} s^{-1}), while that with the somewhat electron-richer acetate ion is noticeably faster ($k \approx 10^8$ dm^3 mol^{-1} s^{-1}; Buxton et al. 1988). Here, it is worth mentioning that in the case of electrophilic/nucleophilic radicals the site of H-abstraction may be reversed, that is, C-H BDE is only one factor that governs the site of attack (Brocks et al. 1998). It has been suggested that a combination of electron donation and electron withdrawal (the push-pull effect) additionally favors H-abstraction (Viehe et al. 1985). Whether this effect or just the contribution of electrophilicity is the reason for the remarkable free-radical chemistry of amino acid anhydrides, is not yet known. In the series of amino acid anhydrides, H-abstraction by •OH occurs at the peptide carbon [cf. reaction (16)], and in alanine anhydride an H-abstraction at the methyl group is not observed. In sarcosine anhydride, however, H-abstraction at the methyl group (22%) is an important process (Mieden and von Sonntag 1989).



Glycine anhydride



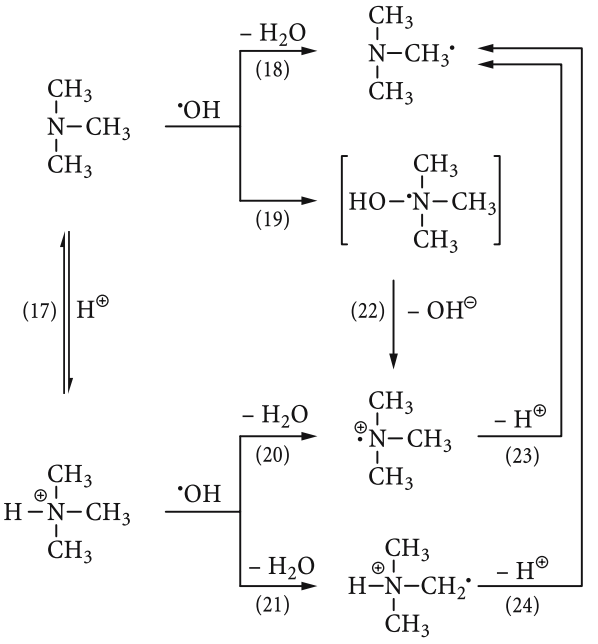
Alanine anhydride



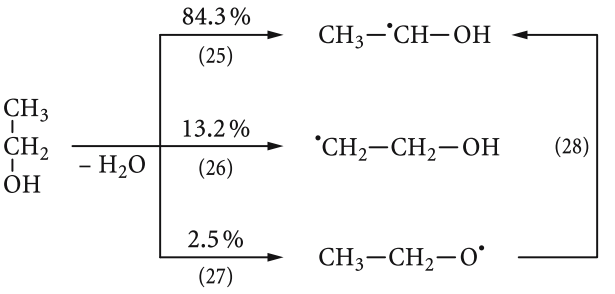
Sarcosine anhydride

Dihydrouracil, an isomer of glycine anhydride, has two kinds of carbon-bound hydrogen atoms. Those activated by the neighboring NH-group react much more readily (90%) than those next to the carbonyl function (ca. 5%; Schuchmann et al. 1984; for details, see Chap. 10). Thus, a high regioselectivity is again observed.

In the case of amines, protonation that withdraws electron density from the center of reaction lowers the rate of reaction by a factor of 30 (Das and von Sonntag 1986). Besides H-abstraction from carbon [reactions (18) and (21)], the formation of *N*-centered radical cations is observed [reactions (19)/(22) and (20); for amino acids see, e.g., Bonifacic et al. 1998; Höbel and von Sonntag 1998]. Reaction (20) is also an H-abstraction reaction. The ET reaction (19)/(22) may proceed via a (bona-fide, very short-lived) adduct (Chap. 7).



In Table 3.2, the rate constants of •OH with MeOH, EtOH, 2-PrOH and *t*BuOH and the percentages of H-abstractions at the various sites [for example, reactions (25)–(28)] are compiled.



Although the rate constants are all close to diffusion controlled, there are noticeable differences. *t*BuOH and MeOH have only primary carbon-bond hydrogens, but despite the fact that *t*BuOH carries three times as many, MeOH reacts faster due to the enhanced radical stabilization by the neighboring OH group (which is equivalent to a lower C–H BDE, Chap. 6). Although a comparison of the yields of α-hydroxyalkyl radicals formed in EtOH and 2-PrOH seems to indicate that the single tertiary hydrogen in 2-PrOH might be as reactive as the two secondary hydrogens in EtOH, a study of the reactions of •OH with D-glucose shows that this must not always be the case, and in this more complex molecule the reactivity of the primary and secondary hydrogens are very similar (Schuchmann

Table 3.2. Rate constants (unit: of $dm^3\ mol^{-1}\ s^{-1}$) of •OH with some alcohols (Buxton et al. 1988) and the position of H-abstraction (in percent). (Asmus et al. 1973)

Alcohol	Rate constant	-CH_3 (%)	-CH_2- (%)	-CH- (%)	-OH (%)
MeOH	9.7×10^8	93	-	-	7.0
EtOH	1.9×10^9	13.3	84.3	-	2.5
2-PrOH	1.9×10^9	13.3	-	85.5	1.2
*t*BuOH	6×10^8	95.7	-	-	4.3

and von Sonntag 1977). In alcohols and carbohydrates, the oxygen-bond hydrogens are quite tightly bond (BDE = 435 kJ mol^{-1}), and hence alkoxyl radicals are formed only in low yields. In water, their detection is complicated due to their ready conversion into α-hydroxyalkyl radicals by a water-assisted 1,2-shift [e.g., reaction (28): Berdnikov et al. 1972; Gilbert et al. 1976; Schuchmann and von Sonntag 1981], and hence their yields given in Table 3.2 might have been slightly underestimated.

The activation energy for H-abstraction from MeOH as measured over a very wide range, from 22–390 °C, has been found to be 13.3 kJ mol^{-1} (Feng et al. 2003). Because of the high reactivity of •OH in its H-abstraction reactions the H/D-isotope effects are rather small, e.g., $k(CH_3OH)/k(CD_3OH) = 2.5$, $k(CH_3CH_2OH)/k(CD_3CD_2OH) = 1.6$ and $k((CH_3)_2CHOH)/k((CH_3)_2CDOH) = 1.5$ (Anbar et al. 1966b). More recently, a value of 1.96 has been reported for the EtOH system (Bonifacic et al. 2003). These values are of interest in comparison with H/D-isotope effects observed for the reaction of •OH with the sugar moiety of DNA (Balasubramanian et al. 1998; Chap. 12).

The rates of •OH addition to C–C double bonds and of H-abstraction are both close to diffusion-controlled. When both reactions can be given by a substrate molecule, addition will be the generally preferred route. This even holds for molecules that have very weakly bound hydrogens. The 1,4- and 1,3-cyclohexadienes provide a good example (Pan et al. 1988). Both contain two C–C double bonds and four weakly-bound pentadienylic hydrogens. In 1,4-cyclohexadiene the two double bonds are separated, while in 1,3-cyclohexadiene the double bonds are conjugated. In the case of 1,4-cyclohexadiene, H-abstraction occurs to an extent of 50%, in the other isomer it is only 25%. Here, •OH addition mainly (50%) yields the allylic radical. Even more pronounced is the situation in the case of toluene. Although there are three weakly bound benzylic hydrogens, H-abstraction occurs with a yield of only 4% (Christensen et al. 1973). Thus, also with thymine, •OH addition to the *C*(5)–*C*(6) double bond is much preferred over an abstraction of a (weakly bound) allylic hydrogen at the *C*(5)–CH_3 group (Chap. 10). This is in marked contrast to, for example, the behavior of peroxyl radicals (Chap. 8).

3.4 Electron Transfer

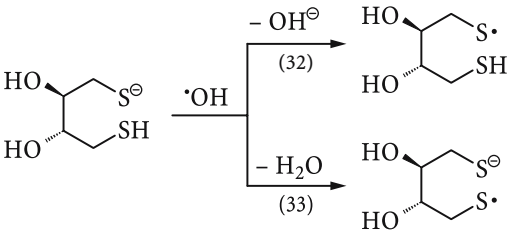
Although the redox potential of $^{\bullet}OH$ is very high [$E(^{\bullet}OH/OH^{-}) = +1.9$ V (Kläning et al. 1985); $E(^{\bullet}OH, H^{+}/H_2O) = 2.73$ (Wardman 1989)], direct ET is rarely observed in $^{\bullet}OH$-reactions, and where it occurs intermediate complexes are likely to be involved. For example, in its reaction with thiocyanate, where the final product is the three-electron bonded dirhodane radical anion [reaction (31); for other three-electron bonded systems, see Chaps. 5 and 7], a similar three-electron bonded intermediate might precede ET [reactions (29) and (30)].

$$^{\bullet}OH + SCN^{-} \rightarrow HOSCN^{\bullet -} \quad (29)$$

$$HOSCN^{\bullet -} \rightarrow OH^{-} + SCN^{\bullet} \quad (30)$$

$$SCN^{\bullet} + SCN^{-} \leftrightarrows (SCN)_2^{\bullet -} \quad (31)$$

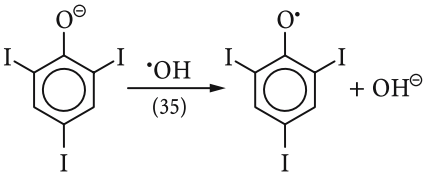
The reaction of $^{\bullet}OH$ with thiolate ions, taken as an overall reaction, is an ET reaction [reaction (36)]. One must, however, again take into account that a three-electron bonded intermediate is formed in the first step (Chap. 7). In semi-deprotonated dithiothreitol reaction (32) dominates over the H-abstraction reaction (33) (Akhlaq and von Sonntag 1987), although the rate constant for the reaction of $^{\bullet}OH$ with a thiol and a thiolate ion are both diffusion controlled ($k = 1.5 \times 10^{10}$ dm^3 mol^{-1} s^{-1}). This is another example of the potentially high regioselectivity of $^{\bullet}OH$ reactions.



Although an ET from phenolates is highly exothermic (for reduction potentials: Lind et al. 1990; Jonsson et al. 1993) and ET is thermodynamically favored over addition (Lundqvist and Eriksson 2000), the usually preferred mode of reaction is addition rather than ET. Yet, addition and ET are in competition (Tripathi 1998), and, when the *ortho*- and the *para*-positions which are the relevant positions of addition for the electrophilic $^{\bullet}OH$ are blocked by a bulky substituent [e.g., reaction (34)] ET may become dominant (Table 3.3). Thus, also for these reactions a short-lived π-complex [cf. reaction (6)] may be postulated as common precursor wherefrom the competition between addition and ET occurs.

Table 3.3. Addition vs. ET in the reactions of •OH with halogenated phenolate ions. (Fang et al. 2000)

Substrate	Addition (%)	Electron transfer (%)
Pentafluorophenolate	73	27
Pentachlorophenolate	47	53
Pentabromophenolate	27	73
2,3,5-Triiodophenolate	3	97



Again, in the oxidation of transition-metal ions, adducts have been established as intermediates [e.g., reaction (35); O'Neill and Schulte-Frohlinde 1975; Asmus et al. 1978; for the equilibrium of Tl^{2+} and •OH, see Schwarz and Dodson 1984].

$$\bullet OH + Tl^{+} \rightarrow TlOH^{+} \tag{35}$$

$$TlOH^{+} + H^{+} \rightarrow Tl^{2+} + H_2O \tag{36}$$

A similar situation holds for the reaction of •OH with Cu^{2+}. The reaction proceeds by the replacement of a water molecule of its solvation shell (Cohen et al. 1990) rather than by ET. In neutral solution, the intermediate formed carries zero charge [reaction (37); Barker and Fowles 1970; Asmus et al. 1978; Ulanski and von Sonntag 2000], and only in more acid solutions more positively charged species start to dominate (Fig. 3.1), but real aqua-Cu^{3+} may not to be formed to any major extent, because at below pH 3 the reaction becomes increasingly reversible [reaction (40); Meyerstein 1971; Ulanski and von Sonntag 2000].

$$\bullet OH + \text{aqua-}Cu^{2+} + 2\,H_2O \rightarrow \text{aqua-}Cu(OH)_3 + 2\,OH^{-} \tag{37}$$

$$\text{aqua-}Cu(OH)_3 + H^{+} \leftrightarrows \text{aqua-}Cu(OH)_2^{+} + H_2O \tag{38}$$

$$\text{aqua-}Cu(OH)_2^{+} + H^{+} \leftrightarrows \text{aqua-}Cu(OH)^{2+} + H_2O \leftrightarrows \text{aqua-}Cu^{2+} + \bullet OH \tag{39/40}$$

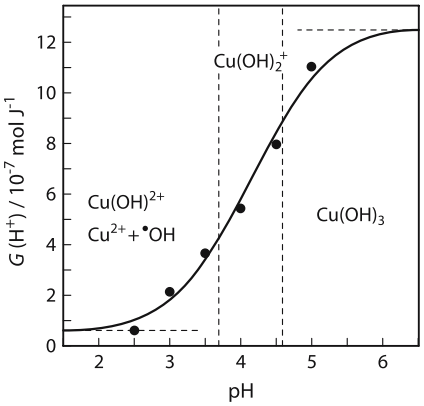


Fig. 3.1. Pulse radiolysis of N_2O-saturated aqueous solutions of Cu^{2+}. Consumption of H^+ as measured by conductometric changes after completion of the reaction. (Ulanski and von Sonntag 2000, with permission)

Thus, in most •OH-induced oxidations short-lived adducts must be considered as intermediates. A case in point in the realm of DNA free-radical chemistry is the oxidation of guanine. From the above, it is evident that •OH, despite its high reduction potential, cannot be directly used for the study of one-electron oxidation reactions. However, one can make use of its high reduction potential by producing other reactive intermediates [e.g., Tl(II); Chap. 10], which no longer undergo an addition to double bonds or H-abstraction.

3.5 Detection of OH Radicals

When one looks for methods to detect •OH, one always has two keep in mind that these radicals are very reactive, and in the presence of substrates their steady-state concentrations are extremely low even at a high rate of •OH production. The fact that •OH only absorbs far out in the UV region (Hug 1981) is thus not the reason why an optical detection of •OH is not feasible. Electron paramagnetic resonance (EPR) must also fail because of the extremely low steady-state concentrations that prevail in the presence of scavengers. The only possibility to detect their presence is by competition of a suitable •OH probe that allows the identification of a characteristic product [probe product, reaction (41)]. When this reaction is carried out in a cellular environment, the reaction with the probe is in competition with all other cellular components which also readily react with •OH [reaction (42)]. The concentration of the probe product is then given by Eq. (43), where [•OH] is the total •OH concentration that has been formed in this cellular environment and η is the yield of the probe product per •OH that has reacted with the probe.

$$^{\bullet}OH + \text{probe} \rightarrow \text{probe product} \tag{41}$$

$$^{\bullet}OH + \text{cellular components} \rightarrow \text{not detectable products} \tag{42}$$

$$[\text{Probe product}] = [^{\bullet}OH] \times \eta \frac{k_{41}[\text{probe}]}{k_{41}[\text{probe}] + k_{42}[\text{cellular components}]} \tag{43}$$

The detection of $^{\bullet}OH$ production in cells is therefore extremely difficult (if not impossible), because both probes and all cell components will react at approximately the same rate, i.e., close to diffusion-controlled (for a detailed discussion of $^{\bullet}OH$-scavenging in cells, see von Sonntag and Schuchmann 1994; von Sonntag et al. 2000). A brief calculation may exemplify this. A cell contains about 70% water and 30% organic material, and thus it is reasonable to take the substrate concentration at about 1 mol dm^{-3} (in subunits in the case of polymers such as DNA). With an averaged $^{\bullet}OH$ rate constant of 10^9 dm^3 mol^{-1} s^{-1}, this yields a scavenging capacity of 10^9 s^{-1} [a somewhat higher scavenging capacity of 3×10^9 s^{-1} was suggested by von Sonntag et al. (2000); a much lower scavenging capacity of 10^7 s^{-1} was estimated by Boveris and Cadenas (1997)]. Although the estimate of 10^9 s^{-1} could be still somewhat on the high side, the value of 10^7 s^{-1} is certainly too low. Yet even with the most reactive probes [$k(^{\bullet}OH$ + salicylate) $\approx 2 \times 10^{10}$ dm^3 mol^{-1} s^{-1}], their scavenging capacity will not exceed 2×10^6 s^{-1} at a probe concentration of 10^{-4} mol dm^{-3} (to achieve this concentration in his body, a human would have to take ~1 g of aspirin). As a consequence, only ~0.2% of $^{\bullet}OH$ (taking 10^9 s^{-1} as the cellular scavenging capacity) that are formed within the cell are scavenged by the probe under such conditions. However, the efficiency factor η is never unity, and in the most commonly used salicylate system it is ca. 0.2 (formation of 2,3-dihydroxybenzoate). Obviously, the possibility of detecting the formation of the probe product very much depends on the sensitivity of its detection. For the salicylate system, very sensitive techniques are available, and the detection limit is given as 2×10^{-7} mol dm^{-3} (Coolen et al. 1998; Tabatabaei and Abbott 1999). Accepting that already a probe product concentration of twice its detection limit (that is, 4×10^{-7} mol dm^{-3} 2,3-dihydroxybenzoate) would give a reliable answer, a total $^{\bullet}OH$ concentration of 2×10^{-3} mol dm^{-3} would have to be formed in the cellular environment. This is quite a high concentration. To visualize this, one may convert it into an equivalent dose of ionizing radiation. Taking 70% cellular water and $G(^{\bullet}OH) = 2.8 \times 10^{-7}$ mol J^{-1}, one arrives at an equivalent dose of 10^4 Gy. This calculation may be taken as a caveat when probing $^{\bullet}OH$ formation in cells, and it really does not matter if the cellular scavenging capacity has been overestimated even by an order of magnitude in this calculation. Thus, detection of $^{\bullet}OH$ formed in cellular systems remains a severe problem (for further details see below), but may be feasible in artificial systems and in areas where the scavenging capacity is markedly lower (extracellular fluids?) and the probe concentration for some reason locally enriched (a suggestion, how this might be tested is made below).

Many detection systems are based on $^{\bullet}OH$-induced hydroxylation of salicylate (see below). Salicylate, however, inhibits some enzymatic reactions that may

be of importance in the in vivo production of •OH. It has, therefore, been suggested (Acworth et al. 1999) to reduce the salicylate concentration, not realizing that a reduction of the probe concentration also reduces its scavenging capacity (Eq. 43).

Some systems that have been proposed as suitable •OH probes (for reviews see Hageman et al. 1992; Kaur and Halliwell 1994, 1996; Loft and Poulsen 1999; von Sonntag et al. 2000), and the principles on which they are based (and, if possible, their reliability) will be discussed in the following.

3.5.1 Aromatic Hydroxylation

Aromatic hydroxylation is most commonly used for the detection of •OH. However, the primary •OH adducts must be oxidized to yield the final product(s). Disproportionation reactions produce these compounds usually only in very low yields. For this reason, an oxidant is required. Although oxygen may serve as an oxidant, the yields are not quantitative because of side reactions (Chap. 8). The addition of a one-electron oxidant, for example $Fe(CN)_6^{3-}$, may overcome this problem (Volkert and Schulte-Frohlinde 1968; Bhatia and Schuler 1974; Madhavan and Schuler 1980; Buxton et al. 1986), but in certain cases an even stronger oxidant such as $IrCl_6^{2-}$ may be required (Fang et al. 1996).

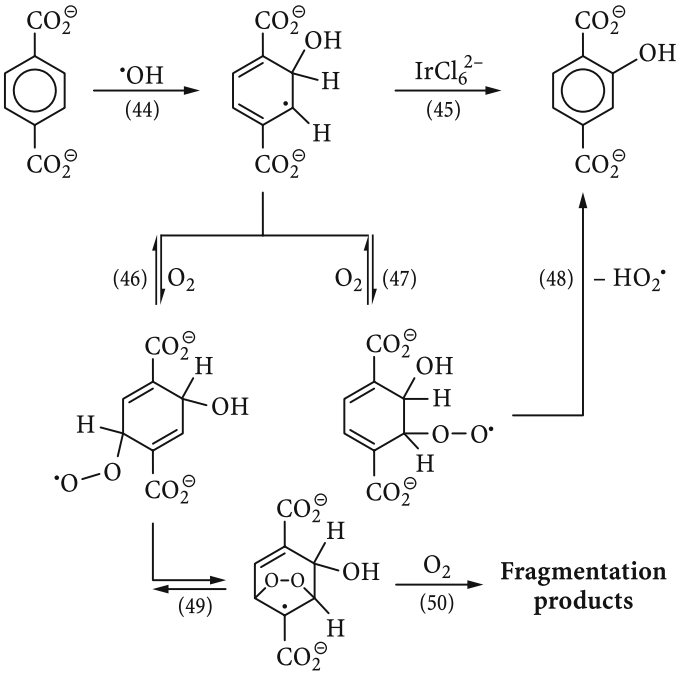
Quite a number of systems that might give rise to •OH-typical products, that is, products that are not formed in enzymatic oxidation processes, have been proposed. For example, phenylalanine (present in all proteins and thus can serve as an internal marker; Karam et al. 1984; Karam and Simic 1988a,b; Kaur et al. 1997), tyrosine (Maskos et al. 1992), terephthalic acid (Armstrong et al. 1963; Matthews 1980), salicylic acid (Ingelmann-Sundberg et al. 1991; Coudray et al. 1995; Bailey et al. 1997) and its isomer 4-hyroxybenzoic acid (Ste-Marie et al. 1996), 5-aminosalicylic acid (Kumarathasan et al. 2001), dopamine (Slivka and Cohen 1985), phthalic hydrazide (Backa et al. 1997), coumarin-3-carboxylic acid (Makrigiorgos et al. 1993, 1995; Chakrabarti et al. 1996, 1998; Manevich et al. 1997; Parker 1998), antipyrine (Coolen et al. 1997) and CO_2 formation as a by-product of the hydroxylation of benzoic acid [Lamrini et al. 1994; reaction (10)]. The very sensitive luminescence detection methods, commonly used in vitro, are not feasible in vivo (Hirayama and Yida 1997). Some of the systems, especially mechanistic implications, will be discussed below in more detail.

It was mentioned above that in aromatic hydroxylation an oxidant is required, and the product yields vary considerably with the oxidant used (for the reason why O_2 does not serve as a typical one-electron oxidant, see Chap. 8). A typical example is the formation of tyrosines from phenylalanine (Table 3.4). Their yields are especially low in the absence of an oxidant, since dimerization usually dominates over disproportionation in these systems. The determination of the products is usually done by either HPLC or GC/MS after trimethylsilylation, and the proteins have to be hydrolyzed prior to analysis. Attention has been drawn to the fact that in vivo cytochrome P-450 enzymes hydroxylate phenylalanine to *p*-tyrosine (Bailey et al. 1997).

Table 3.4. Yields of tyrosines (in % of the •OH yield) formed upon •OH-attack on phenylalamine. (Wang et al. 1993)

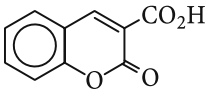
Product	No oxidant (%)	$Fe(CN)_6^{3-}$ (%)	O_2 (%)
Total tyrosine	10	80	52
o-Tyrosine	2.5	37	18
m-Tyrosine	3.5	24	16
p-Tyrosine	4.5	19	18

The terephthalate system follows the same principle. It has the advantage that the main product, 2-hydroxyterephthalate, is the only product which fluoresces and thus can be easily detected, even at low concentrations. Details of the mechanism have been elucidated (Fang et al. 1996). Hydroxyl radicals [k(•OH + terephthalate) = 3×10^9 dm^3 mol^{-1} s^{-1}] react preferentially (85%) at the 2-position [reaction (48)]. The resulting •OH adduct is much more difficult to oxidize than many other hydroxycyclohexadienyl radicals (Chap. 6), and the more powerful oxidant $IrCl_6^{2-}$ is required for a quantitative oxidation [reaction (45)]. With O_2 as the oxidant [reactions (46)–(50)], the yield of 2-hydroxyterephthalate is only

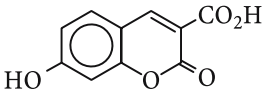


35%. Again, side reactions [reactions (49) and (50)] are the reason for its lower yields (Chap. 8). The detection limit of the fluorescing 2-hydroxyterephthalate has been given as 5×10^{-8} mol dm^{-3} (Saran and Summer 2000).

Aromatic hydroxylation is also the basis of the formation of fluorescent 7-hydroxycoumarine-3-carboxylic acid from coumarine-3-carboxylic acid. Biomolecule-conjugates with coumarin-3-carboxylic acid can be made, and this permits ex vivo probing of •OH in the vicinity of a polymeric conjugate such as proteins or DNA (Makrigiorgos et al. 1993, 1995; Parker 1998; Chakrabarti et al. 1996, 1998; for a review: Makrigiorgos 1999). However, the 7-position in is not an activated position, and hence the yield of 7-hydroxycoumarine-3-carboxylic is very low (~5% of •OH yield; von Sonntag et al. 2000). Despite this fact, this •OH-probing system rates among the most sensitive ones (detection limit 1.5×10^{-8} mol dm^{-3} •OH, taking data reported by Makrigiorgos 1999).



Coumarine-3-carboxylic acid



7-Hydroxycoumarine-3-carboxylic acid

A widely used system is salicylic acid, which was first studied by Grinstead (1960). Being a phenol, the rate of reaction of salicylic acid with •OH is faster [k(•OH + salicylate ion) = 2×10^{10} dm^3 mol^{-1} s^{-1}] than those of the other aromatic acids discussed above. Its main products, 2,3-dihydroxybenzoic acid and 2,5-dihydroxybenzoic acid, are very sensitively determined by HPLC using electrochemical detection (Coolen et al. 1998) or by LC/MS methods (detection limit near 2×10^{-7} mol dm^{-3}; Tabatabaei and Abbott 1999). Due to its phenolic function, the chemistry in the presence of O_2 is different (see below) to that of the other aromatic acids, yielding in the presence of this oxidant high yields of the desired products. The same holds for its isomer, 4-hydroxybenzoic acid. A potential disadvantage is that in a cellular environment salicylic acid may be oxidized by other oxidizing agents.

Since •OH is strongly electrophilic, the OH group directs •OH into its *ortho*- and *para*-positions [reactions (51)–(53)]. One of the *ortho*-positions is occupied by the somewhat bulky carboxylate group which renders reaction (51) less likely than reaction (52). An addition to the *meta*-position can be largely neglected. Upon oxidation of the •OH-adduct radicals, cyclohexadienones are formed [reactions (54)–(56)] which either decarboxylate [reaction (57)] or rearrange into the corresponding phenols [reactions (58) and (59); e.g., Bausch et al. 1976]. Product yields from hydroxybenzoic acids are compiled in Table 3.5; from salicylic acid in Table 3.6.

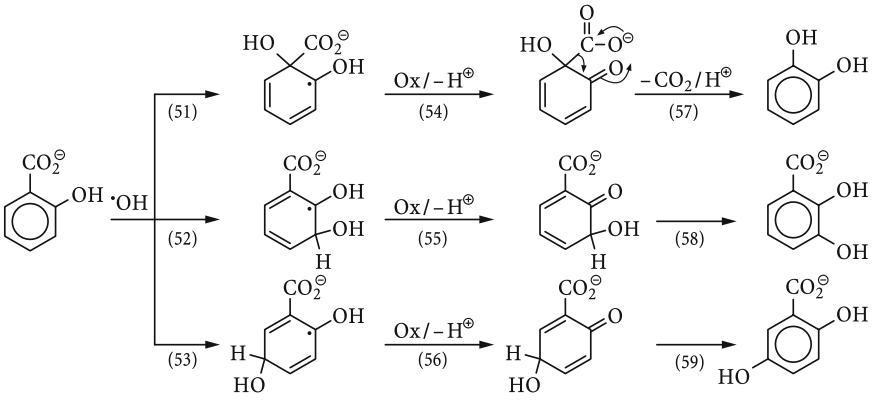
The •OH-adducts of phenols behave differently as compared to those of other aromatic compounds. The parent compound, phenol, has been investigated in quite some detail (Land and Ebert 1967; Raghavan and Steenken 1980; Roder et al. 1999; Mvula et al. 2001). Its *ortho*- and *para*-•OH adducts undergo rapid H^+/OH^--catalyzed water elimination thereby yielding the (thermodynamically

Table 3.5. γ-Radiolysis of hydroxybenzoic acids in N_2O- and air-saturated solutions. *G* values of the dihydroxybenzoic acids (Maskos et al. 1990). Values in parenthesis gives the yield relative to the total •OH yield (N_2O: 5.8×10^{-7} mol J^{-1}, air: 2.9×10^{-7} mol J^{-1})

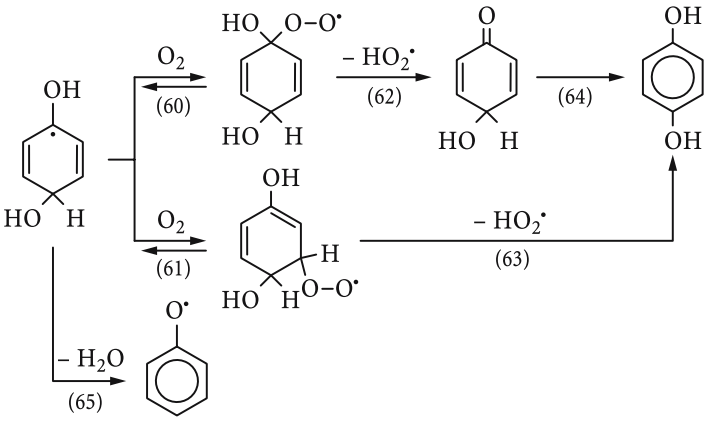
Educt	Gas	2,3-dHB	2,5-dHB	3,4-dHB	2,4-dHB	Total (% •OH)
2-Hydroxy-benzoate	N_2O	1.8	0.2	–	<0.03	2.0 (35%)
	Air	0.83	0.83	–	<0.03	1.7 (59%)
3-Hydroxy-benzoate	N_2O	0.03	0.08	1.02	–	1.13 (19%)
	Air	0.08	0.3	0.73	–	1.25 (43%)
4-Hydroxy-benzoate	N_2O	–	–	0.92	0.02	0.94 (16%)
	Air	–	–	1.33	0.02	1.35 (47%)

Table 3.6. γ-Radiolysis of salicylic acid in N_2O-containing solutions in the presence of $Fe(CN)_6^{3-}$ and O_2. *G* values (unit: 10^{-7} mol J^{-1}) of the products. In parentheses yields in percent of •OH yield. (Mark and von Sonntag 1998, unpubl.)

Product	$Fe(CN)_6^{3-}$	O_2
Catechol	0.5 (9%)	0.8 (14%)
2,3-Dihydroxybenzoic acid	1.5 (26%)	1.5 (26%)
2,5-Dihydroxybenzoic acid	1.3 (22%)	1.7 (29%)
2,4-Dihydroxybenzoic acid	0.1 (2%)	Not detected
Total	3.4 (59%)	4.0 (69%)



more stable; Lundqvist and Eriksson 2000) phenoxyl radical [e.g., reaction (69)]. Interestingly, the *para*-•OH adduct undergoes water elimination much faster than the *ortho*-•OH adduct [$k = \sim 1.7 \times 10^9$ dm^3 mol^{-1} s^{-1} vs $\sim 1.1 \times 10^8$ dm^3 mol^{-1} s^{-1}; Raghavan and Steenken 1980; Roder et al. 1999; Mvula et al. 2001; see also Raghavan and Steenken 1980]. Phosphate buffer catalyses the water elimination in weakly basic solutions with a rate constant of 5×10^7 dm^3 mol^{-1} s^{-1}. At neutral pH, water elimination occurs with $k = 1.8 \times 10^3$ s^{-1} (Raghavan and Steenken 1980; Roder et al. 1999; Mvula et al. 2001) $\sim 5 \times 10^3$ s^{-1} (Land and Ebert 1967). In neutral solutions and the absence of buffer, this reaction is slow compared to the reactions of these •OH adducts with O_2 [e.g., reactions (60) and (61); $k = 1.2 \times 10^9$ dm^3 mol^{-1} s^{-1}; with little if any reversibility, in contrast to other hydroxycyclohexadienyl radicals (Fang et al. 1996); for details see Chap. 8] and the subsequent elimination of $HO_2^\bullet$ [e.g., reactions (62) and (63); $k = 1.3 \times 10^5$ s^{-1}; Raghavan and Steenken 1980; Roder et al. 1999; Mvula et al. 2001]. This reaction is more than 100 times faster than an $HO_2^\bullet$ elimination from the peroxyl radicals of other (nonphenolic) aromatic compounds. This remarkable increase in rate is possibly due to additional routes such as reaction (62). This high rate of $HO_2^\bullet$ elimination renders cyclization and subsequent fragmentation reactions [cf. reactions (49) and (50)] less likely, and in the presence of O_2, the final yields of catechol and hydroquinone are only slightly lower than in the presence of another oxidant (Raghavan and Steenken 1980; Roder et al. 1999; Mvula et al. 2001).



The simple phenol system has been discussed here at some length, because material balance is obtained and mechanistic details are fairly well understood. However, according to the data in Tables 3.5 and 3.6, there is a very noticeable gap in the material balance in the case of the hydroxylated benzoic acids, although some aspects such as the acid-catalyzed water elimination, in salicylate also more pronounced in the case of the *para*-OH-adduct radical, are very similar (Mark and von Sonntag, unpubl.). Interestingly, addition of Fe(III) to oxidize the intermediates also did not improve the material balance (Tables 3.5 and 3.6). From this, it follows that the underlying chemistry of the salicylate and the other hydroxybenzoate systems are at present not yet adequately understood, and the

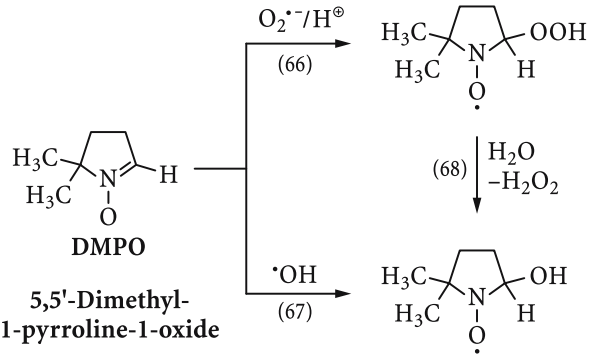
many fluctuations in the reported data may be partly due to the fact that these systems are very sensitive to variations in the reaction conditions. This, however, is not be very helpful for their use as •OH probes.

It is seen from Table 3.5 that in the 2-hydroxybenzoate system, for example, the yields of the 2,3- and 2,5-dihydroxybenzoic acids are close to 1:1 when the precursor radicals are oxidized by O_2, but in the absence of an oxidant this ratio is around 9:1. For the detection of •OH in cellular or in in vivo systems, it is hence not sufficient that these products are formed, but the second requirement is that an adequate oxidant is present (for example, a sufficiently high O_2 tension must be maintained) in order to guarantee that they are formed in a 1:1 ratio. Experiments have been carried out with rats that were given high doses of salicylic acid as a probe for •OH formation induced by the drug (Ste-Marie et al. 1996). Much more 2,5- than 2,3-dihydroxybenzoic acid was detected (cf. Table 3.8). The authors realized that the second requirement of this probe was not fulfilled and stated that there was still the possibility that the drug had induced a metabolic oxidation of salicylic acid yielding mainly 2,5-dihydroxybenzoic acid. Nevertheless, when this paper was later cited in the literature, it was taken as a proof for •OH formation under these conditions. We would like to emphasize here that both requirements, formation of the 2,3- and 2,5-dihydroxybenzoic acids and their 1:1 ratio, is necessary in order to ascertain •OH formation. It is noted that 2,5-dihydroxybenzoic acid is formed in the reaction of salicylic acid with singlet dioxygen ($O_2{}^1\Delta_g$) (Kalyanaraman et al. 1993). More importantly, it may also result from enzyme reactions (Ingelman-Sundberg et al. 1991). Especially the latter makes it difficult if not impossible to ever observe a 1:1 ratio. Whether the suggestion to only concentrate on 2,3-dihydroxybenzoic acid formation for •OH detection (Ingelman-Sundberg et al. 1991) is an adequate solution remains debatable.

3.5.2 Spin Traps

Hydroxyl-radical spin trapping (and detection by EPR) would be a direct detection method [e.g., reaction (67); $k = 2 \times 10^9$ dm^3 mol^{-1} s^{-1}]. Besides reacting with •OH, spin traps also react with $O_2{}^{\bullet-}$ [e.g., reaction (66); $k = 10$ dm^3 mol^{-1} s^{-1}] and the $HO_2{}^\bullet$ adduct to DMPO has only a short lifetime of about 8 min [reaction (68); Pou et al. 1989; Rosen et al. 1994].

Although the difference in rate between •OH and $O_2{}^{\bullet-}$ scavenging by the spin trap is eight orders of magnitude, the yield of the $O_2{}^{\bullet-}$-spin-adduct may be considerably higher, because of the usually much higher steady-state concentration of $O_2{}^{\bullet-}$. In vivo, steady-state concentrations of $O_2{}^{\bullet-}$ have been estimated at around 10^{-11} to 10^{-10} mol dm^{-3} (Boveris and Cadenas 1997) and those of •OH at some 10^{-20} mol dm^{-3} (the latter using too low a scavenger capacity in our opinion, i.e., the •OH steady-state concentration would be at least an order of magnitude lower). As a consequence, its eight orders of magnitude lower rate is more than compensated by the ten (or more) orders of magnitude higher steady-state concentration of $O_2{}^{\bullet-}$. Thus, in a biological system, spin trapping of $O_2{}^{\bullet-}$ will strongly dominate, and the decay of its adduct, reaction (68), may lead to the



erroneous assignment of $^{\bullet}OH$ formation. Moreover, this approach is not directly applicable to the in vivo study of biological oxidations (Kaur and Halliwell 1996), at least not in humans because spin traps are poisonous (Pou et al. 1989). Also, it is considered that the spin-trap method is neither sufficiently sensitive (Mason and Knecht 1994; Rosen et al. 1994), i.e., requiring the presence of micromolar concentrations of the radical species being measured (Ste-Marie et al. 1996), nor practical (Coudray et al. 1995). Thus, it remains to be seen whether a GC/MS technique (Castro et al. 1997) or electrochemical detection (Floyd et al. 1984) can improve the sensitivity situation. The problem of the competition with the $O_2^{\bullet-}$ also seems now on the way to be overcome, and *N*-oxides have been designed that are more specific for $^{\bullet}OH$ (Rosen et al. 1994), and this may be especially true for 2,2-dimethyl-4-methoxycarbonyl-2*H*-imidazole-1-oxide (Tsai et al. 1999). The detection of secondary radicals such as $^{\bullet}CH_3$ from dimethylsulfoxide has been employed to measure $^{\bullet}OH$ (Rosen et al. 1994). Complications can also arise in vivo because the spin trap (such as *N*-oxides) may undergo metabolic reduction (Belkin et al. 1987).

3.5.3 Miscellaneous

There are $^{\bullet}OH$ reactions, however, that allow the detection of the product of the reaction without a further transformation. A case in point is the formation of methanesulfinic acid from DMSO [Babbs and Gale Steiner 1990; reactions (14) and (15)], a nontoxic xenobiotic (Tsay et al. 1998). Methanesulfinic acid has been used as an $^{\bullet}OH$-marker in vivo (Tsay et al. 1998) (detected ex vivo), and ex vivo systems (Fukui et al. 1993). In this context, it may be worth mentioning that methanesulfinic acid can be readily oxidized, and in the presence of O_2 it undergoes a very efficient chain reaction, methanesulfonic acid being the product (Sehested and Holcman 1996; Flyunt et al. 2001). The other product of reaction (15) is the methyl radical. In the presence of O_2, it is converted to a large extent into formaldehyde (Schuchmann and von Sonntag 1984), and it has been suggested (Klein et al. 1981) that this product be used as a marker for $^{\bullet}OH$ in biological DMSO-containing systems.

Table 3.7. Hydroxylation of salicylic acid (5×10^{-3} mol dm^{-3}; product: 2,3-dihydroxybenzoic acid, 2,3-DHB; 2.13×10^{-5} mol dm^{-3} in the absence of a scavenger) and phenylalanine (5×10^{-3} mol dm^{-3}; product: *p*-tyrosine, 7.3×10^{-6} mol dm^{-3} in the absence of a scavenger, total tyrosines 2.21×10^{-5} mol dm^{-3} by the Fenton reagent (Fe(III) 5×10^{-6} mol dm^{-3}, EDTA 1.5×10^{-5} mol dm^{-3}, H_2O_2 5×10^{-5} mol dm^{-3}, ascorbate 5×10^{-5} mol dm^{-3}, phosphate buffer pH 7.4) (Kaur et al. 1997). Calculations are based on competition kinetics using established rate constants. (Buxton et al. 1988)

Scavenger	2,3-DHB measured (%)	2,3-DHB calculated (%)	p-Tyrosine measured (%)	p-Tyrosine calculated (%)
None	100	100	100	100
2-Deoxyribose (0.1 mol dm^{-3})	98	25	69	8
Mannitol (0.1 mol dm^{-3})	28	33	31	11
Formate (0.1 mol dm^{-3})	3	25	46	8

Deoxyribose gives rise to TBA-reactive products (Halliwell 1990; Aruoma 1994; Loft and Poulsen 1999) which can be detected spectrophotometrically or by their fluorescence (Biaglow et al. 1997). However, the test is not specific [the reaction is also given by base propenals in the case of BLM action on DNA which is not •OH-induced (Chap. 12) and can give misleading results (Gutteridge 1986); see also Draper et al. (1993)].

Moreover, the oxidation of methional to ethylene has been proposed (Beauchamp and Fridovich 1970; Biaglow et al. 1997), and the formation of allantoin (Grootveld and Halliwell 1987; Halliwell et al. 1988) from uric acid as well as the imidazolone derivative from histamine (Ching et al. 1995) have been suggested as an index of •OH action in vivo (Halliwell et al. 1988).

3.5.4 References for OH-Radical Probes

For setting up a probe system for use in biological systems, it is required that it provides •OH but also adequately behaves with respect to competition kinetics. The Fenton system seems to fulfill the first criterion in that it produces the required products in good yields but certainly not the second one. As can be seen from Table 3.7, the measured yields and the calculated ones [based on competition kinetics, Eq. (43), and established rate constants] dramatically disagree. The reason for this is not yet known, but it is evident that this system cannot be used with advantage as a reference for •OH production.

Similar effects have been observed when •OH was generated using the xanthine/xanthine oxidase system in the presence of EDTA-complexed iron, a similar Fenton-type system (Owen et al. 1996). The efficiency of suppression of the formation of the dihydroxybenzoic acids by •OH scavengers increases from

mannitol:EtOH:butanol:DMSO in the sequence 1:2:8:24, but it is recalled that their ratios of rate constants with •OH spans a much smaller range (1:1.1:2.5:3.8; Buxton et al. 1988). In addition, the ratio between the products 2,5-dihydroxybenzoate:2,3-dihydroybenzoate changes from 4.8 when the iron is complexed by EDTA to 7.1 when it is complexed by DTPA, and when the complexing agent is omitted only 2,5-dihydroxybenzoate is formed (Owen et al. 1996). In addition, the dihydroxybenzoic acids may interfere in Fenton-type systems and suppress •OH production (Capelle et al. 1992) in a manner which is very far from correlating with their •OH rate constants.

In this context, an interesting observation is worth mentioning. The hydroxylation of salicylic acid by H_2O_2 is catalyzed by fibers (e.g., asbestos and glass wool; Maples and Johnson 1992). The rate of hydroxylation increased proportionally to the H_2O_2 concentration at rather high H_2O_2 concentrations (up to 4×10^{-2} mol dm^{-3}). When the fibers had been treated with salicylate in order to leach out potential transition-metal ions and when, after filtering, this extract was treated with H_2O_2, very little hydroxylation was observed. Although this has been taken as proof that •OH are formed by these fibers, the reaction parameters are difficult to reconcile with what we know at present about Fenton-type reactions, that is, we do not know much about this heterogeneous catalysis of salicylate oxidation. Salicylate is not the only system, where probing •OH scavenging causes considerable difficulties. The luminol/Fe^{2+}/H_2O_2 system, suggested to give adequate data (Hirayama and Yida 1997), also fails when compared with established •OH rate constants.

These dramatic discrepancies mentioned above may also be of some relevance in biological systems, where these probes have been widely used (see below). If •OH were formed in biological systems by Fenton-type reactions, these probe molecules could strongly overestimate (or underestimate) the •OH production in these systems.

A more reliable means of providing a reference of •OH in a biological system may be by means of irradiation with ionizing radiation (von Sonntag et al. 2000). The action of ionizing radiation on an aqueous medium gives rise to •OH whose yield/dose relationship (G value) is known (Chap. 2). Apart from this, since biological media are concentrated solutions the formation of the indicator product, e.g., a phenol (ArOH), via the 'direct effect' [expressions (69) and (70)] must in principle be taken into account as well. It can be shown that with k_{41}[probe]/k_{42}[cellular components] above 10^{-4} the direct effect contributes less than 10% of the •OH pathway.

$$\mathrm{ArH} \rightarrow \mathrm{ArH}^{\bullet+} + \mathrm{e}^- \tag{69}$$

$$\mathrm{ArH}^{\bullet+} + \mathrm{H_2O} \rightarrow \mathrm{ArH(OH)}^{\bullet} + \mathrm{H}^+ \tag{70}$$

The various procedures mentioned above may be calibrated by conducting the incubations for the duration of equal times t and then subject the sample ex vivo to different doses D of ionizing radiation (γ, e-beam). The intrinsic rate of •OH-formation $\nu(\mathrm{OH})_{\mathrm{intrinsic}}$ without irradiation can then be calculated from a

plot of $X_{total}/X_{intrinsic}$ vs D [expression (71)], where X is the quantity that is being experimentally determined.

$$X_{total}/X_{intrinsic} = 1 + G(OH)\, D / t_{incubation} \nu(OH)_{intrinsic} \quad (71)$$

Irradiation would be carried out at the same O_2 status as in incubation, i.e., dose rates will have to be such that O_2 depletion by peroxyl radical formation (Chap. 8) is minimal.

3.5.5 Metabolic OH-Radical Production and Probing for OH Radicals in Vivo

At present, it seems that we do not yet have any reliable data on the rate of metabolic production of $^{\bullet}OH$ within the biological system. This will be proportional to the rate of O_2 uptake. In a human organism, this has been estimated to be near 15 mol day^{-1}, of which 1% is channeled into $O_2^{\bullet-}$, i.e., ~ 4×10^{-3} mol dm^{-3} day^{-1} (Halliwell and Gutteridge 1999). It is not very likely that there is a direct channel into $^{\bullet}OH$, and it is reasonable to assume that its formation requires as precursors $O_2^{\bullet-}$ and H_2O_2 (in combination with low-valent transition metal ions). A major source of H_2O_2 may be $O_2^{\bullet-}$ (cf. the role of SOD). Considering the action of catalase which keeps the cellular steady-state H_2O_2 concentration at a low level, the rate of $^{\bullet}OH$ production must be significantly lower than that of $O_2^{\bullet-}$. Hydroxyl-radical production will not be uniform within the cell, and in a more complex system such as a human it is even more likely that there are areas of low and high $^{\bullet}OH$ production. This will make the probing very difficult, because a given probe may be unevenly distributed within the body.

An approach to estimate the rate of $^{\bullet}OH$ production *in the very neighborhood of DNA* is on the basis of oxidized nucleobases excreted. From that, the total rate of 'oxidative hits' at the DNA in man is estimated at 10^4 per cell per day times 6×10^{13} cells per body (Ames and Shigenaga 1992), i.e., 1×10^{-6} mol $body^{-1}$ day^{-1} (provided that part of the oxidized bases is not recycled in the cell). Putting the ratio of DNA to the total of organic material in the body (without bones) at 100 g/35 kg, we *extrapolate* to 7×10^{-6} mol dm^{-3} day^{-1}. From an estimate of the $^{\bullet}OH$ steady-state concentration (in hepatocytes) (Boveris and Cadenas 1997), one calculates a rate of $^{\bullet}OH$ production of 1.5×10^{-5} mol dm^{-3} day^{-1}.

The two latter values are much below the value of 2×10^{-3} mol dm^{-3} day^{-1} calculated on the basis of salicylate data (Coudray et al. 1995) and the cellular scavenging capacity discussed above. This raises the question whether the salicylate assay can be taken as a reliable in vivo probe. It has been discussed above that 2,5-dihydroxybenzoate is also formed enzymatically and that this product cannot be used for monitoring $^{\bullet}OH$ production. For this reason, reference has always been made only to the other isomer, 2,3-dihydroxybenzoate. However, the question has already been raised whether there are unknown cellular reactions (besides via $^{\bullet}OH$) which produce this isomer (Halliwell and Grootveld 1987). Evidence that this may indeed be the case comes from data compiled in Table 3.8. Here, salicylate (or aspirin) has been given to either a rat or a human,

Table 3.8. Plasma levels of 2,3-dihydroxybenzoate and 2,5-dihydroxybenzoate 2 h after administering salicylic acid (rat; Ste-Marie et al. 1996) or aspirin. (man; Isobe et al. 1996)

Species	mg kg^{-1}	[2,3-DHB]/10^{-9} mol dm^{-3}	[2,5-DHB]/10^{-9} mol dm^{-3}
Rat	300	95	264
Man	10 - 20	63 ± 24	832 ± 309

in the former case at a ~15 times higher concentration. However, close to equal 2,3-dihydroxybenzoate levels were found in the rat and the human despite the fact that in the rat the scavenging capacity of salicylate was about 15 times higher (Eq. 43). Assuming an equal rate of $^{\bullet}OH$ production in these two species, the rat should thus have shown 15 times higher 2,3-dihydoxybenzoate levels. This discrepancy is even larger considering that smaller animals have typically higher metabolic rates and hence a potentially higher rate of free-radical production.

With this cautionary remark in mind, we would like to mention some studies where attempts have been made to monitor $^{\bullet}OH$ production and a variety of conditions, including stressed systems: dextran sulfate-induced colitis (Blackburn et al. 1998), diabetes patients (Ghiselli et al. 1992), obstructive jaundice of rats (Tsay et al. 1998), ischemic reperfused mycocardium (Das et al. 1989), activated human neutrophils (Kaur et al. 1988), healthy humans (Grootveld and Halliwell 1988), fecal flora (Owen et al. 1996), in chondrocytes and cartilage (Tiku et al. 1998).

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4

Hydrogen Atom and Hydrated Electron

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4.1 Some Basic Properties of H• and e_{aq}^-

Much of basic free-radical chemistry of DNA and its constituents have been elucidated with the help of radiation techniques. This requires one to address briefly the properties of the H atom and the hydrated electron, e_{aq}^-, which are important intermediates in the radiolysis of water (Chap. 2.2).

The •OH radical, which is also generated under these conditions, may be converted into •H by reacting it with excess H_2 (Christensen and Sehested 1983). This may require a special pressure cell (Christensen and Sehested 1980). The scavenging of •OH with *t*BuOH ($k = 6 \times 10^8$ dm^3 mol^{-1} s^{-1}) is often the more convenient approach. Under adequate conditions, this leaves H• largely untouched, since its rate of reaction with *t*BuOH is low ($k = 1.7 \times 10^5$ dm^3 mol^{-1} s^{-1}; Buxton et al. 1988; recently revised at 1.15×10^6 dm^3 mol^{-1} s^{-1}; Wojnárovits et al. 2004).

H• is the conjugate acid of e_{aq}^- [pK_a(H•) = 9.1; reactions (1), $k = 2.2 \times 10^7$ dm^3 mol^{-1} s^{-1} and (2), $k = 2.3 \times 10^{10}$ dm^3 mol^{-1} s^{-1} (Buxton et al. 1988), for the thermodynamic properties of this system, see Hickel and Sehested (1985)]. Thus, in pure water, the lifetime of e_{aq}^- is quite long (Hart et al. 1966), even long enough to monitor its presence spectrophotometrically under steady-state ^{60}Co-γ-radiolysis conditions (Gordon and Hart 1964).

$$H^\bullet + OH^- \rightleftharpoons e_{aq}^- + H_2O \quad (1)$$

$$e_{aq}^- + H^+ \rightarrow H^\bullet \quad (2)$$

Reaction (1) is best described as a proton transfer from the weak acid H• to the strong base OH^- (Han and Bartels 1992). For the rapid conversion of e_{aq}^- into H• in neutral solution (i.e., at low H^+ concentration), phosphate buffer may be used [reaction (3); $k = 1.1 \times 10^7$ dm^3 mol^{-1} s^{-1} (Grabner et al. 1973)]. The rate constant depends somewhat on the phosphate concentration, and at 1 mol dm^{-3} phosphate (pH ~5.7) the reported value is 1.85×10^7 dm^3 mol^{-1} s^{-1} (Ye and Schuler 1986).

$$e_{aq}^- + H_2PO_4^- \rightarrow H^\bullet + HPO_4^{2-} \quad (3)$$

The hydrated electron is characterized by its strong absorption at 720 nm ($\varepsilon = 1.9 \times 10^4$ dm^3 mol^{-1} cm^{-1} (Hug 1981); the majority of the oscillator strength is derived from optical transitions from the equilibrated *s* state to the *p*-like excited state (cf. Kimura et al. 1994; Assel et al. 2000). The 720-nm absorption is used for the determination of its reaction rate constants by pulse radiolysis (for the dynamics of solvation see, e.g., Silva et al. 1998; for its energetics see, e.g., Zhan et al. 2003). H• only absorbs in the UV (Hug 1981), and rate constants have largely been determined by EPR (Neta et al. 1971; Neta and Schuler 1972; Mezyk and Bartels 1995) and competition techniques (for a compilation, see Buxton et al. 1988). In many aspects, H• and e_{aq}^- behave very similarly, which made their distinction and the identification of e_{aq}^- difficult (for early reviews, see Hart 1964; Eiben 1970; Hart and Anbar 1970), and final proof of the existence of the

latter was only obtained with the report of its EPR spectrum in frozen alkaline solution (Schulte-Frohlinde and Eiben 1962; Eiben and Schulte-Frohlinde 1965) and the advent of pulse radiolysis (Boag and Hart 1963; Keene 1963, 1964). There is now vast literature on the physical properties and reaction kinetics of e_{aq}^-, and the latter data are only paralleled in number by those of $^{\bullet}OH$ (Buxton et al. 1988).

4.2 Redox Reactions

The hydrated electron is the most powerful reductant ($E_7 = -2.9$ V); $H^{\bullet}$ has a somewhat higher reduction potential ($E_7 = -2.4$ V; for a compilation of reduction potentials, see Wardman 1989). Often, both $^{\bullet}H$ and e_{aq}^- are capable of reducing transition metal ions to their lower oxidation states [e.g., reactions (4) and (5)].

$$Ag^+ + H^{\bullet} \rightarrow Ag + H^+ \tag{4}$$

$$Ag^+ + e_{aq}^- \rightarrow Ag \tag{5}$$

However, there are cases where the reduction potential of $H^{\bullet}$ is insufficient to reduce the metal ion, and the reduction reaction is only given by e_{aq}^- [e.g., reaction (6) (Baxendale and Dixon 1963); for a review see Buxton and Sellers (1977); for a compilation of rate constants of ensuing reactions see Buxton et al. (1995)].

$$Zn^{2+} + e_{aq}^- \rightarrow Zn^+ \tag{6}$$

In strongly acid solution, $H^{\bullet}$ may even react as an oxidant. For example, $H^{\bullet}$ oxidizes Fe^{2+} to Fe^{3+} [reaction (7)]. A hydride, $Fe^{3+}H^-$, is thought to be an intermediate in this reaction.

$$Fe^{2+} + H^{\bullet} + H^+ \rightarrow Fe^{3+} + H_2$$

4.3 Dissociative Electron Capture and Related Reactions

The hydrated electron reacts with many compounds which are capable of releasing an anion by dissociative electron capture [e.g., reaction (8)], and, among others, it was this property which allowed the differentiation between e_{aq}^- and $H^{\bullet}$ [reactions (9) and (10)] (Armstrong et al. 1958; Hayon and Allen 1961; Jortner and Rabani 1962).

$$e_{aq}^- + ClCH_2CO_2H \rightarrow Cl^- + {}^{\bullet}CH_2CO_2H \tag{8}$$

$$H^{\bullet} + ClCH_2CO_2H \rightarrow H_2 + {}^{\bullet}CHClCO_2H \tag{9}$$

The analogous reactions with N_2O (Dainton and Peterson 1962) are commonly used to convert e_{aq}^- into $^\bullet OH$ [reaction (10); $k = 9.1 \times 10^9$ dm^3 mol^{-1} s^{-1} (Janata and Schuler 1982)], since N_2O is largely inert against free-radical attack. Some much slower reactions, often leading to chain reactions, are given by other strongly reducing radicals (Cheek and Swinnerton 1964; Sherman 1967a-c; Ryan and Freeman 1977; Ryan et al. 1978; Al-Sheikhly et al. 1985; Wang et al. 1996).

$$e_{aq}^- + N_2O \rightarrow N_2 + O^{\bullet -} \quad (O^{\bullet -} + H_2O \rightarrow {}^\bullet OH + OH^-) \tag{10}$$

The alternative of using H_2O_2 [reaction (11); $k = 1.3 \times 10^{10}$ dm^3 mol^{-1} s^{-1}] instead of N_2O may lead to complications, since H_2O_2 also reacts with $^\bullet OH$, $^\bullet H$ and many other radicals (Chap. 6).

$$e_{aq}^- + H_2O_2 \rightarrow {}^\bullet OH + OH^- \tag{11}$$

In their reactions with tertiary butylhydroperoxide, $^\bullet H$ and e_{aq}^- show a different selectivity (Phulkar et al. 1990). While $^\bullet H$ undergoes reactions (12) and (13) with about equal probability, i.e., both *t*BuO$^\bullet$ and $^\bullet OH$ are formed, e_{aq}^- yields only *t*BuO$^\bullet$ [reaction (14)]. This preference in splitting the peroxidic bond is due to the much higher solvation energy of the hydroxide compared to the tertiary butoxide ion. For a detailed study on the reaction of $^\bullet H$ with H_2O_2 see Mezyk and Bartels (1995).

$$t\text{BuOOH} + H^\bullet \rightarrow t\text{BuO}^\bullet + H_2O \tag{12}$$

$$t\text{BuOOH} + H^\bullet \rightarrow t\text{BuOH} + {}^\bullet OH \tag{13}$$

$$t\text{BuOOH} + e_{aq}^- \rightarrow t\text{BuO}^\bullet + OH^- \tag{14}$$

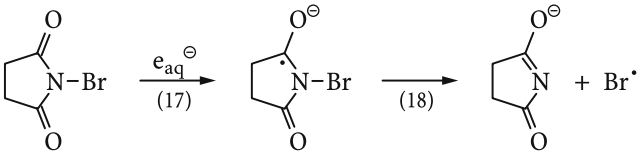
With thiols, e_{aq}^- reacts predominantly by dissociative ET [reaction (15)], but also formation of molecular hydrogen [reaction (16)] is observed (Hoffman and Hayon 1973). As one might expect, there is a considerable drop in the rate constant when the thiol group is deprotonated (for a systematic study, see Mezyk 1995).

$$e_{aq}^- + RSH \rightarrow R^\bullet + SH^- \tag{15}$$

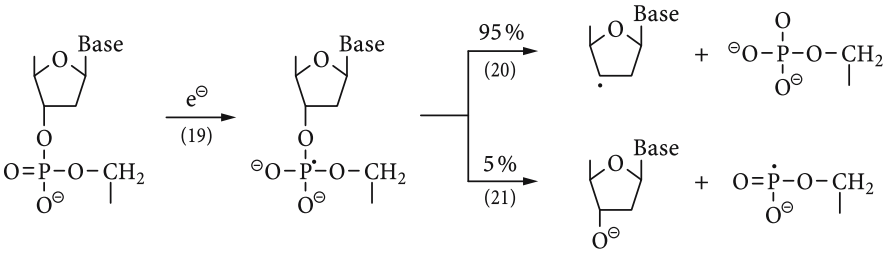
$$e_{aq}^- + RSH \ (+ H^+) \rightarrow RS^\bullet + H_2 \tag{16}$$

In a number of reactions that are written as dissociative electron attachments, short-lived radical anions are in fact intermediates. A case in point is 5BrUra (Chap. 10). An interesting behavior is shown by the radical anion of *N*-bromosuccinimide which does not release a bromide ion but rather fragments into a bromine atom and a succinimide anion [reactions (17) and (18)] (Lind et al. 1991).

The concerted (one-step) dissociative ET is undergone by σ^* electrophores; the ($\sigma^* + \pi^*$) electrophores give rise to two-step processes (Savéant 1993; Schmittel and Ghorai 2001).



The DNA backbone could be split, in principle, by dissociative electron capture [reactions (19)-(21)].



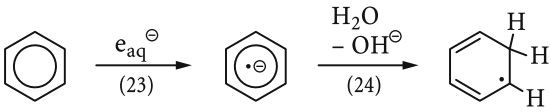
DNA strand breakage by e_{aq}^{-} is not observed in dilute aqueous solution (von Sonntag 1987). DFT calculations show that these reactions are exoenergetic but require activation energy (Li et al. 2003; for quantum-mechanical calculations, see Berdys et al. 2004a; for base radical anions serving as a relay, see Berdys et al. 2004b). It has been envisaged that low-energy-electrons could induce this reaction in competition with other reactions such as thermalization/solvation and addition to the bases (for reviews on the reactions of low-energy electrons in their reactions with DNA, see Sanche 2002a,b). Reactions (19)-(21) have been indeed observed under certain conditions (Becker et al. 2003; Chap. 12). Subexcitation electrons (<3 eV) may also release Thy from DNA (Abdoul-Carine et al. 2004).

4.4 Addition Reactions

Dissociative electron capture can only occur when single-bonds are involved. There are, however, a large number of compounds with high electron affinity such as O_2 and carbonyl, nitro- or cyano-groups containing compounds. With all these compounds, e_{aq}^{-} reacts at diffusion-controlled rates by forming the corresponding radical anion [e.g., reactions (22); $k = 1.9 \times 10^{10}$ dm^3 mol^{-1} s^{-1}]. The radical anions thus-formed are themselves usually strong reductants (including, for example, the Thy radical anion; Chap. 10), and readily hand over an electron to a better electron acceptor (for ketyl radical anions see, e.g., Adams and Willson 1973; for nucleobase radical anions see, e.g., Adams and Willson 1972; Nese et al. 1992; for $O_2^{\bullet -}$ see Chap. 8).

$$e_{aq}^{-} + O_2 \rightarrow O_2^{\bullet -} \qquad (22)$$

Simple olefins do not react with e_{aq}^- at an appreciable rate, but compounds with an extended π-system such as butadiene can also accommodate an additional electron ($k = 8 \times 10^9$ dm^3 mol^{-1} s^{-1}; Hart et al. 1964). However, as in the case of benzene, the rate is often below diffusion controlled [reaction (23); $k = 7.2 \times 10^6$ dm^3 mol;^{1} s;^{1} (Gordon et al. 1977); in THF, the reaction of the solvated electron with benzene is even reversible (Marasas et al. 2003)], and the resulting radical anion is rapidly protonated by water [reaction (24)].



A rapid protonation by water of the electron adducts of spin traps such as DMPO or 2-methyl-2-nitroso-propane yields the same species as are expected for the reaction of H$^\bullet$ (Sargent and Gardy 1975). This prevents a distinction between e_{aq}^- and H$^\bullet$ by using this technique.

H$^\bullet$ readily adds to C–C double bonds. Like $^\bullet$OH, it is a pronounced electrophilic radical ($\rho = -0.45$; Neta 1972) and thus shows a high regioselectivity in its addition reactions. With e_{aq}^-, it shares a fast reaction with O_2 [reaction (25); $k = 1.2 \times 10^{10}$ dm^3 mol^{-1} s^{-1}].

$$H^\bullet + O_2 \rightarrow HO_2^\bullet \qquad (25)$$

4.5 H-Abstraction Reactions

H$^\bullet$ also undergoes H-abstraction reactions, albeit with much lower rates than $^\bullet$OH. This is also reflected in a higher H/D isotope effect [e.g., with 2-PrOH/2-PrOH-d$_2$ $k_H/k_D \approx 7.5$, reactions (26) and (27) (Anbar and Meyerstein 1964); see also Vacek and von Sonntag (1969), vs. $k_H/k_D = 1.5$ for $^\bullet$OH (Anbar et al. 1966)].

$$^\bullet H + HC(CH_3)_2OH \rightarrow H_2 + {}^\bullet C(CH_3)_2OH \qquad (26)$$

$$^\bullet H + DC(CH_3)_2OH \rightarrow HD + {}^\bullet C(CH_3)_2OH \qquad (27)$$

If there is competition between addition and H-abstraction, addition is always preferred. As a consequence, H-abstraction from the sugar moiety is a very minor process in DNA and related compounds (Das et al. 1985).

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Inorganic Radicals

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5.1 General Remarks

Reactive free radicals such as $^{\bullet}OH$ or the e_{aq}^{-} react with many inorganic anions leading to inorganic radicals which have properties not given by the precursor radicals. For this reason, these inorganic radicals have been used with advantage, also in DNA research, to create and study free-radical intermediates otherwise either not accessible or only formed in low yields in competition with other reactions. A typical example is the specific formation of damaged G sites in DNA by using inorganic radicals as the oxidant (Martin and Anderson 1998; Milligan et al. 2000, 2002; Chap. 12.3).

5.2 Formation of Inorganic Radicals and Their Dimeric Radical Anions

Hydroxyl radicals react with many halide (pseudohalide) ions at close to diffusion-controlled rates thereby forming a three-electron-bonded adduct radical [e.g., reaction (1); $k = 1.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1}; Zehavi and Rabani 1972]. These adducts may decompose into OH^- and the halide (pseudohalide) radical which then complexes with another halide (pseudohalide) ion yielding the dihalogen radical anion [reactions (2) and (3); $k_2 = 4.2 \times 10^6$ s^{-1}; $k_3 \approx 10^{10}$ dm^3 mol^{-1} s^{-1}; for resonance Raman spectra of such intermediates, see Tripathi et al. 1985].

$$^{\bullet}OH + Br^- \rightarrow HOBr^{\bullet -} \quad (1)$$

$$HOBr^{\bullet -} \rightarrow Br^{\bullet} + OH^- \quad (2)$$

$$Br^{\bullet} + Br^- \rightleftharpoons Br_2^{\bullet -} \quad (3)$$

They are held together via a weak $\sigma\sigma^*$ three-electron bond. This mechanism adequately describes the reactions of Br^-, I^-, SCN^- and N_3^-. Equilibrium constants are compiled in Table 5.1, where it can be seen that even at moderate halide (pseudohalide) concentrations the equilibrium is shifted to the right [cf. reaction (3); for a direct determination of the forward reaction, see Nagarajan and Fessenden 1985].

A number of mixed complexes have also been characterized (Schöneshöfer and Henglein 1969, 1970; Schöneshöfer 1969, 1973; Ershov et al. 2002). In this context, it is interesting that $Cl^{\bullet}$ also undergoes a weak three-electron bond with water (Sevilla et al. 1997).

Similar hypervalent iodine radicals (9–I–2) are formed in the reaction of alkyl radicals with alkyliodides ($R^{\bullet} + RI \rightarrow R_2I^{\bullet}$), and as an intramolecular complex they are stable enough that a reaction with O_2 is only low (Miranda et al. 2000). Such 9–X–2 radicals have also been postulated as intermediates in the reduction of alkylhalides by α-hydroxyalkyl radicals (Lemmes and von Sonntag 1982).

Table 5.1. Compilation of equilibrium constants of some dimeric radical anions

Dimeric radical anion	Equilibrium constant/dm^3 mol^{-1}	Reference
$Cl_2^{\bullet -}$	6.0×10^4 1.4×10^5	Buxton et al. (1998); Yu and Barker (2003)
$Br_2^{\bullet -}$	3.9×10^5	Liu et al. (2002)
$I_2^{\bullet -}$	1.1×10^5	Baxendale et al. (1968); Schwarz and Bielski (1986)
$(SCN)_2^{\bullet -}$	2×10^5	Baxendale et al. (1968); Buxton and Stuart (1995)
$N_6^{\bullet -}$	0.33	Alfassi et al. (1986)

In basic and neutral solutions, $Cl^\bullet$ is a stronger oxidant than $^\bullet OH$ (cf. Table 5.2), and the formation of $Cl_2^{\bullet -}$ only proceeds in acid solution [reactions (4) and (5); Anbar and Thomas 1964]. Details of this very complex situation and the involvement of equilibrium (6) have been redetermined (Buxton et al. 1998). It is evident that the even more strongly oxidizing fluorine atom cannot be produced this way.

$$^\bullet OH + Cl^- \rightleftharpoons HOCl^{\bullet -} \quad (4)$$

$$HOCl^{\bullet -} + H^+ \rightarrow H_2O + Cl^\bullet \quad (5)$$

$$Cl^\bullet + Cl^- \rightleftharpoons Cl_2^{\bullet -} \quad (6)$$

An exception is the reaction of $^\bullet OH$ with the cyanide ion. Its $^\bullet OH$ adduct rapidly protonates even at high pH, but in this reaction the cyanide radical is not formed because of its very high reduction potential (Wardman 1989). It rather undergoes an enol→keto tautomerization [overall reaction (7); Behar and Fessenden 1972; Behar 1974; Büchler et al. 1976; Bielski and Allen 1977; Muñoz et al. 2000].

$$^\bullet OH + CN^- + H_2O \rightarrow {}^\bullet C(O)NH_2 \quad (7)$$

Since bicarbonate/carbonate are omnipresent, it is of special interest that $^\bullet OH$ also reacts with these ions, yielding the oxidizing $CO_3^{\bullet -}$ radical [reactions (8 and 9); $k_8 = 3.9 \times 10^8$ dm^3 mol^{-1} s^{-1}, $k_9 = 8.5 \times 10^6$ dm^3 mol^{-1} s^{-1}; Buxton and Elliot 1986], i.e. bicarbonate is about 40 times less reactive.

$$^\bullet OH + CO_3^{2-} \rightarrow OH^- + CO_3^{\bullet -} \quad (8)$$

$$^{\bullet}OH + HCO_3^- \rightarrow H_2O + CO_3^{\bullet -} \quad (9)$$

The $CO_3^{\bullet -}$ radical is characterized by a strong absorption at 600 nm ($\varepsilon \approx 2000$ dm^3 mol^{-1} cm^{-1}) (Weeks and Rabani 1966; Zuo et al. 1999). This absorption does not change between pH 0 and 13 (Czapski et al. 1999; Zuo et al. 1999; see, however, Eriksen et al. 1985). Nevertheless, the pK_a value of $HCO_3^{\bullet}$ [equilibrium (10)] continues to be debated, and pK_a values between 7 and 9.6 have been reported (Chen and Hoffman 1972; Chen et al. 1973; Eriksen et al. 1985; Zuo et al. 1999). However, there is now increasing evidence (Bisby et al. 1998; Czapski et al. 1999) that it must be much lower, <0 (Czapski et al. 1999), and hence it is more acidic than its parent [p$K_a(H_2CO_3) = 3.5$]. For a detailed discussion, see Czapski et al. (1999).

$$HCO_3^{\bullet} \rightleftharpoons H^+ + CO_3^{\bullet -} \quad (10)$$

The reaction of $^{\bullet}OH$ with nitrite yields $^{\bullet}NO_2$ [reaction (11); $k \approx 1 \times 10^{10}$ dm^3 mol^{-1} s^{-1}], but also the reaction of e_{aq}^- with nitrate leads to $^{\bullet}NO_2$ via a short-lived adduct, $NO_3^{\bullet 2-}$ [reaction (12); $k = 9.7 \times 10^9$ dm^3 mol^{-1} s^{-1}]. The latter subsequently decays into $^{\bullet}NO_2$ [reaction (13); $k = 4.6 \times 10^3$ s^{-1}; Alfassi et al. 1998].

$$^{\bullet}OH + NO_2^- \rightarrow OH^- + {}^{\bullet}NO_2 \quad (11)$$

$$e_{aq}^- + NO_3^- \rightarrow NO_3^{\bullet 2-} \quad (12)$$

$$NO_3^{\bullet 2-} + H_2O \rightarrow {}^{\bullet}NO_2 + 2OH^- \quad (13)$$

The sulfate radical anion, $SO_4^{\bullet -}$, can be formed from peroxodisulfate, $S_2O_8^{2-}$, photolytically [reaction (14)] or by its reaction with e_{aq}^- [reaction (15); $k = 1.2 \times 10^{10}$ dm^3 mol^{-1} s^{-1}] and $^{\bullet}H$ [reaction (6); $k = 1.4 \times 10^7$ dm^3 mol^{-1} s^{-1}]. For the photolytic generation, one has to take into account that peroxodisulfate absorbs only weakly in the UV with absorption coefficients very close to that of H_2O_2 (Chap. 2.4). However, its decomposition can be sensitized by triplet acetone (acetone reacts only slowly with $SO_4^{\bullet -}$). As measured by photoacoustic calorimetry, the reaction volume and enthalpy changes for reaction (14) are 8.9 ml mol^{-1} and 120 kJ mol^{-1}, respectively (Brusa et al. 2000). Compared to H_2O_2, peroxodisulfate has a rather weak O–O bond, and this is reflected by its ready cleavage which can also be induced thermally (Strasko et al. 2000) at temperatures, where H_2O_2 does not yet show any noticeable decomposition (Chap. 2.4).

$$S_2O_8^{2-} + h\nu \rightarrow 2SO_4^{\bullet -} \quad (14)$$

$$S_2O_8^{2-} + e_{aq}^- \rightarrow SO_4^{\bullet -} + SO_4^{2-} \quad (15)$$

$$S_2O_8^{2-} + {}^{\bullet}H \rightarrow SO_4^{\bullet -} + HSO_4^- \quad (16)$$

The $SO_4^{\bullet -}$ radical is one of the strongest oxidants (cf. Table 5.2), and in the presence of Cl^- it is in equilibrium with $Cl^{\bullet}$ [reaction (17); $K = 2.9$ (Buxton et al. 1999), $K = 1.5$ (Yu et al. 2004)].

$$SO_4^{\bullet -} + Cl^- \rightleftharpoons SO_4^{2-} + {}^{\bullet}Cl \quad (17)$$

The phosphate radical, $PO_4^{\bullet 2-}$, is related to $SO_4^{\bullet -}$. It may be similarly generated photolytically or radiolytically from peroxodiphosphate (Maruthamuthu and Neta 1977, 1978; Maruthamuthu 1980; Kumar and Adinarayana 2000). Its reduction potential is lower than that of $SO_4^{\bullet -}$, that is, the latter reacts with phosphate, although the rate of reaction is only slow (with HPO_4^{2-} $k = 1.2 \times 10^6$ dm^3 mol^{-1} s^{-1}, with $H_2PO_4^-$ $k < 7 \times 10^4$ dm^3 mol^{-1} s^{-1}; Maruthamuthu and Neta 1978). Its reactions are of some interest in the context of DNA free-radical chemistry, since in DNA this type of radical may be formed upon oxidation of the phosphate groups, for example, by ionizing radiation (direct effect) or photoionization at short wavelengths.

The $H_2PO_4^{\bullet}$ radical has pK_a values of 5.7 and 8.9, and the oxidation power decreases in the order $SO_4^{\bullet -} > H_2PO_4^{\bullet} > HPO_4^{\bullet -} > PO_4^{\bullet 2-}$ (Maruthamuthu and Neta 1978). The $H_2PO_4^{\bullet}$ radicals abstract H-atoms at slightly higher rates than $SO_4^{\bullet -}$, and in their addition reactions they are similarly electrophilic ($\rho = -1.8$) as the $SO_4^{\bullet -}$ radical (Maruthamuthu and Neta 1977).

When generating the di(pseudo)halide radical anions radiolytically, one has to keep in mind that the halide ions do not react with $^{\bullet}H$, but HN_3/N_3^- does. Originally, it has been suggested that $N_3^{\bullet}$ and H_2 are formed (Alfassi et al. 1986), but it was later shown that it reacts according to reaction (18) (Deeble et al. 1990).

$$^{\bullet}H + N_3^- + H^+ \rightarrow {}^{\bullet}NH_2 + N_2 \quad (18)$$

The $SeO_3^{\bullet -}$, a Se(V) species with a high redox potential (cf. Table 5.2) can be produced radiolytically from Se(VI) upon reduction by e_{aq}^- [reaction (19)] and from Se(IV) by $^{\bullet}OH$ [reaction (20); Kläning and Sehested 1986].

$$SeO_4^{2-} + e_{aq}^- \rightarrow SeO_3^{\bullet -} \quad (19)$$

$$SeO_3^{2-} + {}^{\bullet}OH \rightarrow SeO_3^{\bullet -} + OH^- \quad (20)$$

The $SeO_3^{\bullet -}$ radical has been used with advantage to oxidize DNA specifically at G sites (Martin and Anderson 1998; Milligan et al. 2002).

The strongly oxidizing $^{\bullet}NO_3$ radical ($E^0 = 2.0$ V vs. SCE in acetonitrile) can be generated photolytically in acetonitrile [reaction (21)].

$$(NH_4)_2Ce(NO_3)_6 + h\nu \rightarrow {}^{\bullet}NO_3 + (NH_4)_2Ce(NO_3)_5 \quad (21)$$

It has been used to study the oxidative cleavage of Thy dimers (Krüger and Wille 2001; Chap. 10.14).

All these radicals have oxidizing properties, but there are also some inorganic radicals which have reducing properties ($E < 0$ V; cf. Table 5.2).

For example, H-abstraction from formate [reaction (22)], addition of $^{\bullet}OH$ to CO [reaction (23)] or the reaction of e_{aq}^- with CO_2 [reaction (24)] yields the (reducing) $CO_2^{\bullet -}$ radical. The pK_a value of its conjugate acid $^{\bullet}CO_2H$ [equilibrium (25)] continues to be in dispute. Using different approaches to determine its pK_a,

Table 5.2. Compilation of the reduction potentials of some inorganic radicals; values selected by Wardman (1989). For further data, see also Das et al. (1999)

Couple	E/V
$F^{\bullet}/F^{-}$	+3.60
$^{\bullet}OH, H^{+}/H_2O$	+2.73
$Cl^{\bullet}/Cl^{-}$	+2.60
$SO_4^{\bullet-}/SO_4^{2-}$	+2.47
$Cl_2^{\bullet-}/2Cl^{-}$	+2.30
$Br^{\bullet}/Br^{-}$	+2.00
$^{\bullet}OH/OH^{-}$	+1.90
$O_3, H^{+}/HO_3^{\bullet}$ (pH 7)	+1.80
$SeO_3^{\bullet-}/SeO_3^{2-}$ (pH 7)	+1.77
$Br_2^{\bullet-}/2\ Br^{-}$	+1.60
$CO_3^{\bullet-}/CO_3^{2-}$	+1.59
$I^{\bullet}/I^{-}$	+1.40
$HO_2^{\bullet}, H^{+}/H_2O_2$ (pH 0)	+1.48
$(SCN)_2^{\bullet-}/2SCN^{-}$	+1.33
$N_3^{\bullet}/N_3^{-}$	+1.30
$^{\bullet}SH/SH^{-}$	+1.15
$O_3/O_3^{\bullet-}$ (pH 11-12)	+1.01
$I_2^{\bullet-}/2I^{-}$	+1.05
$NO_2^{\bullet}/NO_2^{-}$	+1.00
$HO_2^{\bullet}/HO_2^{-}$	+0.79
$O_2(^1\Delta_g)/O_2^{\bullet-}$	+0.65
$O_2/O_2^{\bullet-}$	−0.33*
$CO_2/CO_2^{\bullet-}$	−1.90
aq/e_{aq}^{-}	−2.87

* This reduction potential relates, by definition, to O_2-saturated solutions. For comparison with other values that are based on molarity, a value of −0.179 V should be taken (Wardman 1991).

values of 3.9 (Fojtik et al. 1970), 2.3 (Flyunt et al. 2001), 1.4 (Buxton and Sellers 1973) and −0.4 (Jeevarajan et al. 1990) are reported in the literature. The reason for these large discrepancies is not yet known. Nevertheless, the majority of these values suggest that $^{\bullet}CO_2H$ is more acidic than its parent, formic acid (pK_a = 3.75).

$$^{\bullet}OH\ (H^{\bullet}) + HCO_2^- \rightarrow H_2O\ (H_2) + CO_2^{\bullet -} \quad (22)$$

$$^{\bullet}OH + CO \rightarrow {}^{\bullet}CO_2H \quad (23)$$

$$e_{aq}^- + CO_2 \rightarrow CO_2^{\bullet -} \quad (24)$$

$$^{\bullet}CO_2H \rightleftharpoons CO_2^{\bullet -} + H^+ \quad (25)$$

In radiolytic studies, $CO_2^{\bullet -}$ is often used with some advantage as a precursor of the superoxide radical, $O_2^{\bullet -}$ [reaction (26); Chap. 8.4].

$$CO_2^{\bullet -} + O_2 \rightarrow CO_2 + O_2^{\bullet -} \quad (26)$$

5.3 Reduction Potentials of Inorganic Radicals

The redox properties of the inorganic radicals (for a compilation see Table 5.2) have been widely used to produce specifically certain radicals, notably radical cations and radical anions. It is worth mentioning that $^{\bullet}OH$, although it has a high redox potential, normally undergoes addition rather than one-electron transfer (ET) reactions (Chap. 3).

5.4 Reactions of Inorganic Radicals with Organic Substrates

In DNA free-radical chemistry, the strongly oxidizing radicals, notably $SO_4^{\bullet -}$, $Br_2^{\bullet -}$ and $N_3^{\bullet}$, and transition-metal ions in high oxidation states, such as Tl^{2+}, have often been used to produce one-electron-oxidized intermediates (Chap. 10.2). These inorganic radicals react very rapidly with many organic substrates by forming adduct radicals. In the case of $SO_4^{\bullet -}$, for example, an adduct to a C–C double bond may precede ET. In fact, in the reaction with simple olefins, such adduct radicals have been detected by EPR (Davies and Gilbert 1984). They also may form adducts via $\sigma\sigma^*$ three-electron bonds, notably at sulfur and even nitrogen. Typical examples are the oxidation of thiolates by $Br_2^{\bullet -}$ or $I_2^{\bullet -}$ forming $RSBr^{\bullet -}$ ($RSI^{\bullet -}$) as short-lived intermediates (Packer 1984) or with sulfides such as methionine (Hiller and Asmus 1981; Champagne et al. 1991). In proteins, $Br_2^{\bullet -}$ has been used to study the transformation of the methionyl

radical into the tyrosyl radical (Prütz et al. 1985a), and such an adduct must be formed in the first step.

Although $Br_2^{\bullet-}$ has a higher redox potential than $N_3^{\bullet}$, it reacts notably slower (Neta et al. 1988). An explanation for this is provided by the Marcus theory for outer-sphere ET (Marcus 1993, 1999). According to this theory, the rate constant for ET between two redox partners, **A** and **B**, is given approximately by the simple expression $k = (k_{ex,A} \times k_{ex,B} \times K_{A,B})^{1/2}$, where $k_{ex,A}$ and $k_{ex,B}$ are the rate constants for self-exchange for the two redox couples and $K_{A,B}$ is the equilibrium constant of the redox reaction. Clearly, for a common redox couple **A**, the rate constant will increase with increasing $K_{A,B}$, i.e., increasing redox potential of redox couple **B** and also with increasing $k_{ex,B}$. The value of k_{ex} for $Br_2^{\bullet-}/2Br^-$ is so much smaller than that for $N_3^{\bullet}/N_3^-$ that this effect outweighs by far the reverse effect of $K_{A,B}$. In addition, $N_3^{\bullet}$ appears in many cases to oxidize by way of an inner-sphere ET, which further increases the rate beyond what the Marcus theory would predict. As for experimental values for k_{ex} of main-group redox couples, only very few of them are known. One such couple is $O_2^{\bullet-}/O_2$ for which k_{ex} has been determined to be 450 ± 160 (Lind et al. 1989). However, employing certain molecular parameters, such as bond lengths, vibration frequencies, ionic radii, etc., more or less accurate values for k_{ex} can be predicted by use of the Marcus theory.

The $^{\bullet}NO_2$ radical (and also the $CO_3^{\bullet-}$ radical) are of some biological interest (Augusto et al. 2002) because they play some role in the reactions of peroxynitrite (Chap. 2.4). For example, $^{\bullet}NO_2$ oxidizes tyrosine to nitrotyrosine (Prütz et al. 1985b), and the latter has been considered a promoter of free-radical damage in DNA model systems (Prütz 1986). In this context, it may be of interest that $CO_3^{\bullet-}$ reacts with a self-complementary ODN ($k = 1.9 \times 10^7$ dm^3 mol^{-1} s^{-1}) exclusively at G (by ET) (Chap. 11.2).

The dihalogen radical anions are electrophilic radicals [a correlation with the σ values of aromatic compounds gives $\rho = -1.5$ for $Cl_2^{\bullet-}$ (Hasegawa and Neta 1978) and $\rho = -1.1$ for $Br_2^{\bullet-}$ (Kemsley et al. 1974)]. The temperature dependence of the rates of reaction of these and other inorganic radicals have been measured (Alfassi et al. 1990). The rates of reaction do not seem to correlate with the exothermicity of the reactions. The variations in the rate constants appear more strongly dependent on changes in the pre-exponential factors rather than on changes in the activation energy.

Although the dihalogen radical anions mainly act as oxidants, in the reaction of $Br_2^{\bullet-}$ with phloroglucinol as much as 8% bromination has been observed (Wang et al. 1994), and in the reaction of $Cl_2^{\bullet-}$ with fumaric acid the chlorine atom adduct has been detected by EPR (Chawla and Fessenden 1975). When their rate constant with a given substrate is low, it is also possible that the observed products are due to the reaction of the halogen atom which is always in equilibrium with dihalogen radical anion. A case in point may be the reaction of $Br_2^{\bullet-}$ with the pyrimidines (Cadet et al. 1983) and the reactions of $Cl_2^{\bullet-}/Cl^{\bullet}$ with *t*BuOH (Mertens 1994), where the observed rate of reaction is very low (Hasegawa and Neta 1978), and even with benzene under certain conditions (Alegre et al. 2000; for a discussion of chlorine atom reactions in organic solvents, see Ingold et al. 1990).

With alcohols, the $SO_4^{\bullet-}$ radical reacts by H-abstraction rather than by ET (Eibenberger et al. 1978). These reactions are rather slow (e.g., with *t*BuOH $k = 8 \times 10^5\ dm^3\ mol^{-1}\ s^{-1}$; Redpath and Willson 1975; Buxton et al. 1999), and thus $SO_4^{\bullet-}$ is considerably more selective than $^{\bullet}OH$ (Gilbert et al. 1999). $SO_4^{\bullet-}$ is always generated from $S_2O_8^{2-}$, and like H_2O_2 this peroxide readily reacts with reducing radicals such as derived from primary and secondary alcohols thereby inducing chain reactions with complex kinetics (Schuchmann and von Sonntag 1988; Ulanski and von Sonntag 1999) but also with those derived from the pyrimidine nucleobases (Chap. 10.2).

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Carbon-Centered Radicals

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6.1 General Remarks

The carbon-centered radicals that one deals with in the free-radical chemistry of DNA are not simple alkyl radicals but carry substituents that determine the reactivity of the radical. The property of a radical (e.g., its redox property) may change considerably when a substituent is protonated or deprotonated. For this reason, the pK_a values of radicals are discussed first. Besides redox-reactions, H-transfer reactions play an important role. In the free-radical chemistry of DNA, such H-transfer processes can even cause two opposite effects: repair by an external H-donor such as a thiol and damage amplification by intramolecular H-transfer. Addition of carbon-centered radicals to C=C double bonds is a well-established process and technically widely exploited (cf. radical-induced polymerization), but it will be shown that an addition to C=N double bonds occurs as well. This reaction is of quite some importance in purine free-radical chemistry. The reverse of the addition reaction is the homolytic β-fragmentation. When adequately substituted, carbon-centered radicals also undergo heterolytic β-fragmentation, and in DNA such a reaction leads to strand breakage and a short-lived radical cation. The strong oxidative power of the radical cation can amplify the damage by oxidizing a neighboring G. Details of the DNA-related free-radical chemistry are discussed in Chapter 12, but the fundamental aspects are discussed here. Most carbon-centered radicals react with O_2 at close to diffusion-controlled rate forming peroxyl radicals. Thus, in a natural environment the slower ones of those free-radical reactions that are discussed in the present chapter will be interfered with by O_2, and peroxyl radicals will be formed. The chemistry of peroxyl radicals is discussed in Chapter 8. There in now a wealth of rate constants of the reactions of carbon-centered radicals available (for a compilation see Asmus and Bonifacic 1984; Beckwith et al. 1984; for the chemistry undergone by radical ions see, e.g. Schmittel and Ghorai 2001).

6.2 pK_a Values of Radicals

Most of the pK_a values of free radicals have been determined by pulse radiolysis, and it is therefore useful to recall, how fast pK equilibria become established. In general, the reaction of H^+ with an acid anion is practically diffusion-controlled [reaction (2); k ranging between 5×10^9 dm^3 mol^{-1} s^{-1} and 5×10^{10} dm^3 mol^{-1} s^{-1} (Eigen et al. 1964; Perrin et al. 1981)]. The same holds for the deprotonation of an acid by OH^- [reaction (3)]. The rates of reaction (4) can be calculated from the pK_a value taking into account that $K_w = [H^+] \times [OH^-] = 10^{14}$ mol^2 dm^{-6}.

$$AH \rightarrow A^- + H^+ \quad (1)$$

$$A^- + H^+ \rightarrow AH \quad (2)$$

$$AH + OH^- \rightarrow A^- + H_2O \quad (3)$$

$$A^- + H_2O \rightarrow AH + OH^- \quad (4)$$

Thus, the pK_a values of an acid is only determined by its rate of dissociation [reaction (1)]. Hence, in a homologous series, electron-withdrawing substituents reduce the pK_a value by rendering the environment of the acidic function more positive and thereby accelerating the rate of dissociation of the (positively charged) proton. In contrast, an electron-donating substituent increases the pK_a value.

The time required to reach equilibrium very much depends on the pK_a value of the acid. An acid with a pK_a value of 4, for example, deprotonates with a rate of ~10^6 s^{-1}. Thus, the equilibrium is established within a few microseconds. On the other hand, an acid with a pK_a value of 7 dissociates with a rate of ca. 10^3 s^{-1}, and the equilibrium becomes established only on the millisecond time range. In a pulse radiolytic experiment, a large part of the radicals will thus have disappeared in bimolecular termination reactions, before an equilibrium is reached. Buffers speed-up the protonation/deprotonation reactions, and their addition can overcome this problem. Yet, they deprotonate acids and protonate their corresponding anions typically two to three orders of magnitude more slowly than OH^- and H^+ (for a DNA-related example, see Chap. 10.4; for potential artifacts in the determination of pK_a values using too low buffer concentrations, see, e.g., von Sonntag et al. 2002).

Rapid protonation is not restricted to carboxylate ions, but is generally valid for other heteroatom atoms such as oxygen, nitrogen and sulfur in alcohols, amines and thiols. Also in these reactions, no rehybridization and skeletal rearrangements reduces the rate below diffusion-controlled. On the other hand, such factors play a major role when carbanions are protonated, and the rate of their protonation is generally considerably slower. The same factors reduce the rate of deprotonation of acidic C–H groups. For example, the pK_a value of barbituric acid is 4.0. In this acid, the keto/enol equilibrium (5) is largely on the side of the keto form which dissociates with a rate of only 6.9×10^3 s^{-1} despite its low pK_a value (Eigen et al. 1965; Koffer 1975; Schuchmann and von Sonntag 1982).

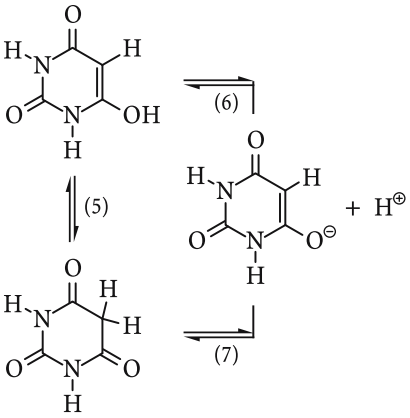


Table 6.1. pK_a values of some radicals in comparison with those of their parent compounds, wherefrom these radicals may be formed by H-abstraction. For comparison, the pK_a values of some radicals are also given which can only produced by electron addition and subsequent protonation (indicated with an asterisk)

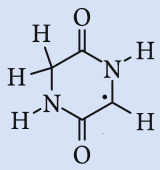
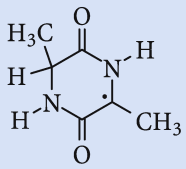
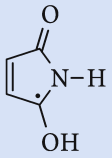
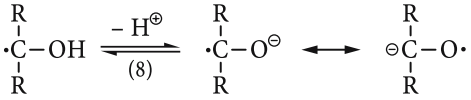
Radical	pK_a	Parent compound	ΔpK_a	Reference
$^{\bullet}CH_2OH$	10.71 10.7	MeOH	−4.38	Laroff and Fessenden (1973) Asmus et al. (1966)
$^{\bullet}CH(CH_3)OH$	11.51 11.6	EtOH	−4.42	Laroff and Fessenden (1973) Asmus et al. (1966)
$^{\bullet}C(CH_3)_2OH$	12.03 12.2	2-PrOH	−5.07	Laroff and Fessenden (1973) Asmus et al. (1966)
$^{\bullet}C(CF_3)_2OH$	1.70	Hexafluoro-2-PrOH	−8.1	Laroff and Fessenden (1973)
$^{\bullet}CH(OH)_2$	9.5	Formaldehyde hydrate	−3.8	Stockhausen and Henglein (1971)
$^{\bullet}CO_2H$	2.3	Formic acid	−1.45	Flyunt et al. (2001)
$^{\bullet}CH_2C(O)OH$	4.8 4.2	Acetic acid	0 −0.6	Neta et al. (1969) Schuchmann et al. (1989)
$^{\bullet}CH(NH_3^+)CONH_2$	4.3	Glycinamide	−3.6	Rao and Hayon (1975)
	9.8	Glycine anhydride	−12.2	Mieden and von Sonntag (1989) Muñoz et al. (2000)
	10.6	Alanine anhydride	–	Mieden and von Sonntag (1989)
$^{\bullet}CH_2$-CH=C(OR)OH	5.5	Acrylate*	–	Takács et al. (2000)
	6.9	Thy*	–	Hayon (1969)

Table 6.1. *Continued*

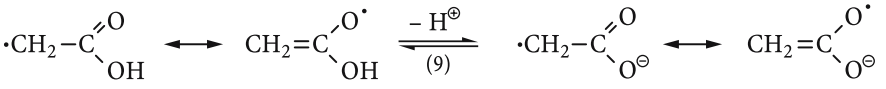
Radical	pK_a	Parent compound	ΔpK_a	Reference
	2.8	Maleimide*	–	Hayon and Simic (1972) von Sonntag et al. (2002)

This general phenomenon is also of some importance in the case of the protonation of radical anions which can protonate at a heteroatom or at carbon. Kinetically, protonation at the heteroatom is always faster even when protonation at carbon is thermodynamically favored. A case in point is the protonation of the Thy radical anion (Chap. 10.4).

α-Hydroxyalkyl radicals are less solvated than their parent alcohols due to a lower polarity of the C-O bond and lower H-bond acceptor ability of the oxygen atom (Block et al. 1999). They are considerably more acidic than their parent alcohols by four or more p*K* units (Table 6.1). The same effect is observed even more dramatically the case of the cyclic dipeptides (Merenyi et al. 1986; Mieden and von Sonntag 1989). This increase in acidity is possibly largely due to an increase in mesomery such as shown in reaction (8), and captodative substituent effects (Viehe et al. 1985) seem to contribute further in the case of the peptide radicals.



Interestingly, the pK_a values of acetic acid and its corresponding radical are practically identical (Table 6.1; Neta et al. 1969; Schuchmann et al. 1989, see also Schuchmann et al. 2000). In this case, not only the anion, but also the acid experiences a gain in mesomery [reaction (9)].

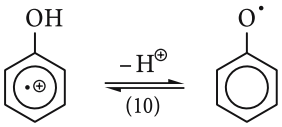


There is a continuing discussion as to the pK_a value of $^{\bullet}CO_2H$ radical. In the literature, values are found ranging from –0.2 (Jeevarajan et al. 1990), 1.4 (Buxton and Sellers 1973), 2.3 (Flyunt et al. 2001) to 3.9 (Fojtik et al. 1970). Why the value

of 2.3 has been chosen here (Table 6.1), has been discussed in the given reference.

Methyl substitution increases the electron density at the neighboring carbon due to hyperconjugation effects and thus shifts the pK_a of the radical to higher values as it does with the parent compound (Table 6.1).

As expected, radical cations may have especially low pK_a values due to their positive charge. A good example is phenol (pK_a = 10) whose radical cation has a pK_a value of –2 (Dixon and Murphy 1976). Here, the difference with respect to its parent is as large as 12 pK units [equilibrium (10)].



(10)

Similar effects are observed with the nucleobase-derived radical cations (Chap. 10.2).

6.3 Oxidation of Carbon-Centered Radicals

Reduction potentials of radicals may be determined by pulse radiolysis (Chap. 13.3) or photomodulated voltammetry (Wayner and Houman 1998; for a compilation, see Steenken 1985; Wardman 1989).

Carbon-centered radicals which are substituted by electron-donating groups such as -OR or -NR_2 are readily oxidized. A convenient one-electron oxidant is $Fe(CN)_6^{3-}$. For example, it oxidizes α-hydroxyalkyl or α-alkoxyalkyl radicals at practically diffusion-controlled rates ($k \approx 2 \times 10^9$ dm^3 mol^{-1} s^{-1}; Adams and Willson 1969). Substitution by electron-donating groups that are not capable of rapid deprotonation, such as -OCH_3, stabilizes the resulting carbocation. They then can have a considerable lifetime in water (Steenken et al. 1986b) while the lifetime of the parent, $C_2H_4^{\bullet+}$, is only ~100 fs (Mohr et al. 2000).

In the case of α-hydroxyalkyl radicals, the corresponding carbonyl compounds are formed in full yields. In contrast, the oxidation of α-alkoxyalkyl radicals by $Fe(CN)_6^{3-}$ may not always be a straightforward outer-sphere ET reaction (Janik et al. 2000a,b). Details are as yet not fully understood.

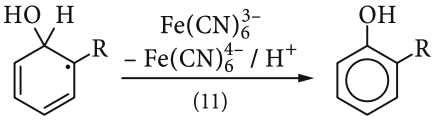
In studies on the •OH-induced aromatic hydroxylation, the oxidation of hydroxycyclohexadienyl radicals by $Fe(CN)_6^{3-}$ has often been used for the determination of the yield of a given precursor radical (Volkert et al. 1967; Volkert and Schulte-Frohlinde 1968; Klein et al. 1975). Other oxidants such as Cu^{2+}, Ag^+, Fe^{3+} or Cr^{3+} give lower yields, and complications are apparent, since, for example, the oxidation potential of Ag^+ (0.8 V) is higher than that of $Fe(CN)_6^{3-}$ (0.36 V; Bhatia and Schuler 1974). The substituent has a strong influence on the rate of oxidation (Table 6.2), and quantitative oxidation to the corresponding phenol [reaction (11)] is only observed with electron-donating substituents (Buxton et al. 1986). Even the terephthalate ion •OH-adduct requires the stronger oxidant

Table 6.2. Rate constants of the oxidation of hydroxycyclohexadienyl radicals by $IrCl_6^{2-}$ and $Fe(CN)_6^{3-}$ (unit: 10^9 dm^3 mol^{-1} s^{-1})

Substrate	$IrCl_6^{2-}$	$Fe(CN)_6^{3-}$	Reference
Anisol	3.1	2.3	Buxton et al. (1986)
Acetylaniline		1.5	Buxton et al. (1986)
Toluene	3.0	0.16/0.019[a]	Buxton et al. (1986)
Benzene	3.1	0.015	Buxton et al. (1986)
Benzene-d_6		0.012	Buxton et al. (1986)
Fluorobenzene	2.4	0.01	Buxton et al. (1986)
Chlorobenzene	1.6	0.0055	Buxton et al. (1986)
Benzonitrile	0.45	<0.0002	Buxton et al. (1986)
Terephthalate	0.077		Fang et al. (1996)

[a]Rates of oxidation of two different isomers

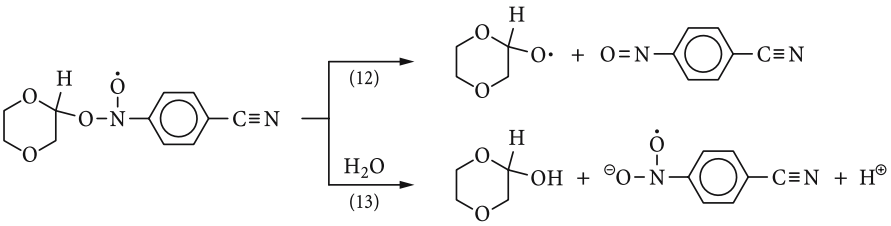
$IrCl_6^{2-}$ for complete (i.e., sufficiently rapid with respect to the bimolecular decay of the radicals) oxidation (Fang et al. 1996).



(11)

In these oxidation processes, often the kinetically favored rather than the thermodynamically favored product is formed. A case in point is the oxidation of pyrimidine-6-yl radicals by $Fe(CN)_6^{3-}$ to an isopyrimidine which is only a short-lived intermediate that results in the formation of the pyrimidine and its hydrate (Chap. 10.3).

In pulse radiolysis experiments, TNM is often preferred as an oxidant over $Fe(CN)_6^{3-}$ because the strongly absorbing nitroform anion is formed which can be used to determine the yield of reducing radicals (Chap. 10.3). TNM is only capable of oxidizing strongly reducing radicals, but with less reducing radicals an adduct may be formed which usually absorbs at shorter wavelengths and has a lower absorption coefficient (Schuchmann et al. 1995). In fact, the formation of adducts is common in the oxidation of radicals by nitro compounds. These adducts may decompose into two directions [e.g., reactions (12) and (13)] (Nese et al. 1995).



In rare cases, the one-electron oxidized products are also readily oxidized, and the three-electron oxidized product is observed. A case in point is the oxidation of the 4-chlorobenzyl radicals by $Fe(CN)_6^{3-}$ to the corresponding benzaldehyde. The 4-chlorobenzylalcohol is not the intermediate that is further oxidized by $Fe(CN)_6^{3-}$, and thus the mechanism of the formation of 4-chlorobenzaldehyde is rather complex (Merga et al. 1996). Since $Fe(CN)_6^{3-}$ is commonly used as a simple and effective oxidant also in DNA free-radical chemistry, such potential complexities have to be kept in mind.

Although α-hydroxyalkyl radicals such as the hydroxymethyl radical are oxidized without an adduct being noticed [reaction (14); $k = 1.6 \times 10^8$ dm^3 mol^{-1} s^{-1}], such a complex becomes apparent in the case of β-hydroxyalkyl radicals [reactions (15) and (16); $k_{15} = 3 \times 10^7$ dm^3 mol^{-1} s^{-1}; $k_{16} = 330$ s^{-1}; Freiberg and Meyerstein 1980], whereby the epoxide is formed (Söylemez and von Sonntag 1980).

$$^{\bullet}CH_2OH + Cu^{2+} \rightarrow Cu^{+} + CH_2O + H^{+} \quad (14)$$

$$Cu^{2+} + {}^{\bullet}CH_2\text{-}CH_2OH \rightarrow Cu\text{-}CH_2\text{-}CH_2OH^{2+} \quad (15)$$

Although α-hydroxyalkyl radicals are good reducing agents their reduction po-

$$Cu{-}CH_2{-}CH_2{-}OH^{2\oplus} \xrightarrow[(16)]{} H_2C(O)CH_2 + Cu^{\oplus} + H^{\oplus}$$

tential is often not sufficiently low to undergo electron transfer reactions. However, they might do so, when they are deprotonated. A case in point is the reaction of the hydroxymethyl radical with nitrous oxide [reaction (17)] (Wang et al. 1996; for other reducing radicals that undergo chain reactions with nitrous oxide, see Sherman 1967a-c; Al-Sheikhly et al. 1985).

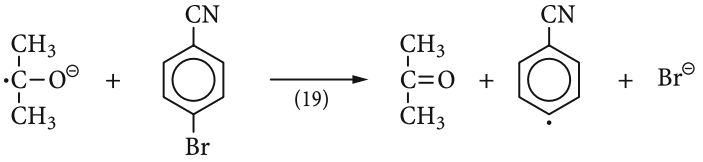
$$^{\bullet}CH_2O^{-} + N_2O \rightarrow CH_2O + N_2 + O^{\bullet -} \quad (17)$$

The reduction of disulfides is also only given by the hydroxymethyl radical anion [reaction (18)] while the hydroxymethyl radical itself is practically unreactive (Akhlaq et al. 1989).

$$RSSR + {}^{\bullet}CH_2O^{-} \rightarrow RS^{-} + RS^{\bullet} + CH_2O \quad (18)$$

The 2-hydroxy-2-propyl radical anion is a stronger reductant than the hydroxymethyl radical anion. Thus, a number of reactions are readily given by the for-

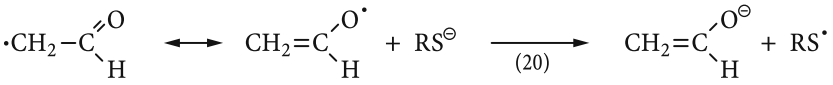
mer, while the latter remains unreactive. For example, only 2-hydroxy-2-propyl radical anion transfers electron to 4-bromobenzonitrile thereby inducing an efficient chain reaction. The ET reaction (19) is so fast that it is not the rate-limiting step in this chain reaction (Fang et al. 1997).
Typically, the reactivity of the halogen substituent follows the sequence I > Br > Cl (Lemmes and von Sonntag 1982), and three-electron-bonded adducts to



the halogen are potential intermediates (for similar intermediates see Chapts 5.2 and 7.4). This type of reaction is also given by 5IUra and 5BrUra (Chap. 10.7).

6.4 Reduction of Carbon-Centered Radicals by Electron Transfer

In the reduction of radicals by ET, simple carbanions are practically never formed, and one-electron reduction of a carbon-centered radicals is only effective if the electron can be accommodated by the substituent, e.g., a carbonyl group [reaction (24), whereby upon electron transfer the enolate is formed (Akhlaq et al. 1987)]. Thus, in their reduction reactions these radicals react like heteroatom-centered radicals despite the fact that major spin density is at carbon.
The mesomeric forms of the pyrimidine *C*(6)-adduct radicals may be written with the free spin at a heteroatom and hence have as oxidizing properties.



Their yields have been determined with the help of a strong reductant, TMPD (Chap. 10.3). Other convenient probes for monitoring oxidizing radicals are, for example, ABTS and ascorbate (Wolfenden and Willson 1982; Bahnemann et al. 1983).

Radical cations are especially strong oxidants, since they are highly electron deficient. They are intermediates in •OH-induced DNA strand breakage and are capable of oxidizing a neighboring G (Chaps 12.4 and 12.9).

Table 6.3. Compilation of some R-H BDEs (unit: kJ mol^{-1})

R–H bond	BDE/kJ mol^{-1}	Reference
C-H (aromatic)	465	Berkowitz et al. (1994)
C-H (vinylic)	465	Berkowitz et al. (1994)
C-H (primary)	423	Berkowitz et al. (1994)
C-H (primary in MeOH)	402	Berkowitz et al. (1994)
C-H (secondary)	412	Berkowitz et al. (1994)
C-H (secondary in EtOH)	396	Reid et al. (2003)
C-H (tertiary)	403	Berkowitz et al. (1994)
C-H (tertiary in 2-PrOH)	393	Reid et al. (2003)
C-H (allylic)	367	Berkowitz et al. (1994)
C-H (benzylic)	367	Berkowitz et al. (1994)
C-H (pentadienylic)	343	Schöneich et al. (1990)
C-H (in peptides)	330-370	Reid et al. (2003)
O-H (alcoholic)	436	Berkowitz et al. (1994)
O-H (water)	499	Berkowitz et al. (1994)
O-H (phenolic)	360	Parsons (2000)
O-H (hydroperoxidic)	366	Golden et al. (1990)
S-H (thiolic)	366	Armstrong (1999)
S-H (thiophenolic)	330	Armstrong (1999)

6.5 H-Transfer Reactions

The C-H bond dissociation energies (BDEs) decrease in the sequence vinylic ≈ phenylic > primary > secondary > tertiary > allylic > pentadienylic ≈ benzylic considerably (Table 6.3).

R-H BDEs energies are certainly one parameter that has to be taken into account when discussing H-transfer reactions, yet many other aspects have to be considered to account for the kinetics of such a reaction (for the calculation of activation energies in H-abstraction reactions, see Zavitsas and Chatgilialoglu 1995).

Table 6.4. Compilation of the H-abstraction rate constants (unit: $dm^3\ mol^{-1}\ s^{-1}$) of some radicals from alcohols in aqueous solution at room temperature

Radical	Substrate	Rate constant	Reference
Uracil-5-yl	2-PrOH	4.1×10^7	Mertens and von Sonntag (1994)
Phenyl	2-PrOH	1.2×10^7	Mertens and von Sonntag (1994)
Vinyl	2-PrOH	2×10^5	Mertens and von Sonntag (1994)
Trichlorovinyl	*t*BuOH	1.6×10^5	Mertens and von Sonntag (1994)
	MeOH	6.3×10^6	Mertens and von Sonntag (1994)
	EtOH	2.4×10^7	Mertens and von Sonntag (1994)
	2-PrOH	4.1×10^7	Mertens and von Sonntag (1994)
Methyl	MeOH	220	Thomas (1967)
	EtOH	590	Thomas (1967)
	2-PrOH	3400	Thomas (1967)
2-Hydroxypropyl	2-PrOH	430	Burchill and Wollner (1972)
2-Hydroxy-2-methyl-propyl	2-PrOH	~700	Schuchmann H-P and von Sonntag (1988)

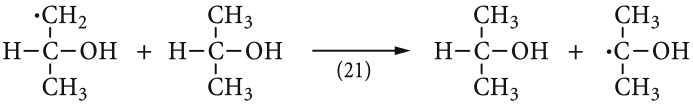
For inactivation of reactive free radicals by thiols and the repair of DNA radicals (Chaps 11.2 and 12.10), it is of relevance that the RS-H bond is especially weak and renders thiols good H-donors. Although phenols and hydroperoxides have also low O-H BDEs, they are rather poor H-donors in water. For example, the rate of H-abstraction of $^{\bullet}CH_3$ from H_2O_2 is 1000-fold lower in water than in the gas phase (Ulanski et al. 1999; see also below). H-abstraction from O-H is strongly influenced by the solvent (Das et al. 1981), and the variation of the rate constant of H-abstraction from phenols and *tert*-butylhydroperoxide by the cumyloxyl radical with the solvent was interpreted in terms of hydrogen-bonding between solvent and phenol, i.e., the stronger the hydrogen bonding the lower the rate constant (Avila et al. 1995; for kinetic solvent effects in H-abstraction reactions see also Valgimigli et al. 1995; Banks et al. 1996; Snelgrove et al. 2001).

It is important to note that the rate of reaction of alkyl radicals with thiols does not simply correlate with the exothermicity of the reaction, i.e., with the BDE of the C-H bond to be formed. For example, the tertiary 2-hydroxypropyl radical reacts more readily with thiols than the primary hydroxymethyl radical, and this reacts even faster than the methyl radical (Table 6.4). The reason for this surprising behavior has been discussed in terms of the charge and

spin polarization in the transition state, as determined by AIM analysis and in terms of orbital interaction theory (Reid et al. 2002).

The C-H BDE in peptides is even lower than that of the S-H BDE in thiols as a consequence of the exceptional stability of the radical products due to captodative stabilization (Viehe et al. 1985; Armstrong et al. 1996). Yet, the observed rate constants for the reaction of $^{\bullet}CH_3$ and $^{\bullet}CH_2OH$ with, e.g., alanine anhydride are markedly slower than with a thiol. This behavior has been discussed in terms of the charge and spin polarization in the transition state, as determined by AIM analysis, and in terms of orbital interaction theory (Reid et al. 2003). With respect to the 'repair' of DNA radicals by neighboring proteins, it follows that the reaction must be slow although thermodynamically favorable.

Although BDE is by far not the only factor that determines the kinetics of H-transfer reactions, within a given series of simple alkyl radical a correlation seems to hold (Table 6.4). In polymers, where the lifetime of the polymer-bound radicals may be long, radical transfer reactions by intramolecular H-abstraction (primary → secondary → tertiary) are common (Chap. 9.4). In general, whenever a system starts with a mixed radical system (e.g., in the reaction of $^{\bullet}OH$ with 2-PrOH: 2-hydroxy-prop-2-yl and 2-hydroxypropyl) a steady-state is approached which is dominated by the lower-energy radical [here: 2-hydroxy-prop-2-yl, cf. reaction (21)]. This process is favored by low initiation rates and high substrate concentrations, and these two factors determine whether such an H-transfer manifests itself is also in the final products.



As far as DNA is concerned, the most weakly bound hydrogen is the allylic hydrogen of the methyl group in Thy. For dGuo, the sequence of the C-H BDE has been calculated as C(1′) < C(4′) < C(3′) < C(2′) < C(5′) (Table 6.5). In DNA, accessibility as determined by the given structure often overruns factors that are connected with the R-H BDE (Chap. 12.2).

In DNA, an H-transfer from the methyl group in Thy and from the sugar moiety to DNA radicals may occur. A well-documented radical that is capable of reacting with the sugar moiety is the uracil-5-yl radical formed upon photolysis and radiolysis of 5BrUra-containing DNA (Chaps 10.7 and 12.6). From Table 6.4, it is seen that this radical is indeed very reactive and thus this kind of H-abstraction is not unexpected. Steric conditions permitting, it will abstract any hydrogen from the sugar moiety. Less reactive radicals will undergo such a reaction not only more slowly but also much more selectively (note, for example, the high kinetic isotope effects of such reactions; $^{\bullet}CH_3$ + CH_3OH/CD_3OH: k_H/k_D = 8.2 at 150 °C in the gas phase; Gray and Herod 1968). In DNA, an H-transfer from the sugar moiety to a base radical has never been proven with certainty, but in model systems such as poly(U) and poly(C) it is quite evident (Chap. 11.2). Here, H-transfer is believed to occur from *C*(2′), a position that in the *ribo*-polynucleotides is activated by the neighboring OH group. In DNA, the corresponding hydrogen is bound more strongly.

Table 6.5. Calculated bond dissociation energies (BDE) of the various C–H bonds in the sugar moiety of dGuo. (Steenken et al. 2001)

C–H bond	BDE/kJ mol^{-1}
C(1′)	367
C(2′)	399
C(3′)	385
C(4′)	378
C(5′)	386

It has been mentioned above that the weakest C-H bond in DNA is that of the methyl group in the Thy moiety. In Thd, this position is attacked, albeit slowly, even by peroxyl radicals (Martini and Termini 1997), although they have only a low H-abstractive power (cf. the low BDE of ROO-H; Table 6.3). That this may also hold for DNA in vivo may be deduced from the observation that one of the corresponding oxidation products is excreted into the urine in rather large amounts (Chap. 12).

The (oxidizing) α-carboxyalkyl radicals do not react readily with thiols (Table 6.4). They are, however, rapidly reduced by thiolate ions [reaction (20)]. The reactions of thiols with DNA radicals play a very important role in the chemical repair of DNA radicals in cells (Chaps 12.10 and 12.11). The reversibility of the H-donation of thiols, that is, H-abstraction by thiyl radicals, is discussed in Chapter 7.4.

6.6 Conversion of Reducing Radicals into Oxidizing Ones (Umpolung)

There are a number of reactions which change the redox property of the radical (Umpolung). A typical, DNA-relevant, case of Umpolung is the buffer-catalyzed conversion of the reducing Thy electron adduct protonated at oxygen into the oxidizing carbon-protonated tautomer (Chap. 10.3). Another example is the proton-catalyzed conversion of the (reducing) •OH-adduct at C(5) in pyrimidines to the oxidizing C(6) •OH-adducts (Chap. 10.3).

These reactions are real tautomerization reactions, but the quite common water elimination reactions can also completely change the redox property of a radical. A case in point is the radical derived from ethylene glycol which is a reducing α-hydroxyalkyl radical which is transformed by water elimination into the fomylmethyl radical (see below) whose oxidizing property has been discussed above [reaction (20)]. Similarly, the phenol •OH-adduct is a reason-

ably good reductant, while the phenoxyl radical formed upon water elimination (Chap. 3.4) has oxidizing properties. Similar reactions play an important role in purine free-radical chemistry (Chap. 10.3).

6.7 Reactions with Hydrogen Peroxide

Hydrogen peroxide is rather unique in so far as it can act as an oxidant as well as an H-donor. This is exemplified its reaction with the hydroxymethyl radical. In this case, ET [reaction (22); $k = 6 \times 10^4$ dm^3 mol^{-1} s^{-1}] is faster than H-abstraction [reaction (32); $k = 2.75 \times 10^3$ dm^3 mol^{-1} s^{-1} (Ulanski and von Sonntag 1999)].

$$^{\bullet}CH_2OH + H_2O_2 \rightarrow CH_2O + H^+ + OH^- + {}^{\bullet}OH \quad (22)$$

$$^{\bullet}CH_2OH + H_2O_2 \rightarrow CH_3OH + HO_2^{\bullet} \quad (23)$$

While the former induces a chain reaction, the latter terminates the chain. The basic form of the hydroxymethyl radical, $CH_2O^{\bullet-}$ [pK_a($^{\bullet}CH_2OH$) = 10.8], is a stronger reducing agent [reaction (24); $k = 4 \times 10^5$ dm^3 mol^{-1} s^{-1}], and the chain length increases with increasing pH.

$$CH_2O^{\bullet-} + H_2O_2 \rightarrow CH_2O + OH^- + {}^{\bullet}OH \quad (24)$$

At very high pH, H_2O_2 also dissociates [pK_a(H_2O_2) = 11.6]. Although the hydroxymethyl radical anion undergoes the H-abstraction even faster [reaction (25), $k = 2.9 \times 10^4$ dm^3 mol^{-1} s^{-1}] the chain length comes to a halt, because the anion of H_2O_2 is no longer a good electron acceptor.

$$CH_2O^{\bullet-} + HO_2^- \rightarrow CH_3O^- + O_2^{\bullet-} \quad (25)$$

The methyl radicals can only undergo the H-abstraction reaction, and since the C-H BDE is higher by ca. 38 kJ mol^{-1} in methane than in MeOH (McMillen and Golden 1982; Golden et al. 1990; Berkowitz et al. 1994), the rate of reaction (26) is higher ($k = 2.7 \times 10^4$ dm^3 mol^{-1} s^{-1}; Ulanski et al. 1999) than that of reaction (23).

$$^{\bullet}CH_3 + H_2O_2 \rightarrow CH_4 + HO_2^{\bullet} \quad (26)$$

However, compared to the gas phase ($k_{26} = 3.3 \times 10^7$ dm^3 mol^{-1} s^{-1}; Tsang and Hampson 1986), the reaction is three orders of magnitude slower. A similar situation is found for $^{\bullet}OH$ [reaction (27); k(in water) $= 2.7 \times 10^7$ dm^3 mol^{-1} s^{-1} (Buxton et al. 1988); k(gas phase) $= 1 \times 10^9$ dm^3 mol^{-1} s^{-1} (Baulch et al. 1984)]. In water, a low rate constant has also been found for the reaction of $^{\bullet}OH$ with tertiary butylhydroperoxide [reaction (28); $k = 10^7$ dm^3 mol^{-1} s^{-1}; (Phulkar et al. 1990)].

$$^{\bullet}OH + H_2O_2 \rightarrow H_2O + HO_2^{\bullet} \quad (27)$$

$$^{\bullet}OH + (CH_3)_3COOH \rightarrow H_2O + (CH_3)_3COO^{\bullet} \quad (28)$$

The reasons for this dramatic drop in the rate of reaction on going from the gas phase to aqueous solution have been discussed above. It may be recalled that the difference in the exothermicity of reactions (23) and (26) is as much as 38 kJ mol^{-1}. The relatively small effect of the thermochemistry on the rate can be rationalized by invoking the polar effect in the transition state (Russell 1973; Pross et al. 1991; Roberts 1996). Quantum mechanical studies on the solvent effect on the rate of the $^{\bullet}CH_3$ plus H_2O_2 system can reproduce the dramatic drop upon going from the gas phase to aqueous solutions and indicate that the major reason is the difference between the solvation energies of H_2O_2 and $HO_2^{\bullet}$ in water (Delabie et al. 2000).

The rate constants that we are concerned with here are quite moderate for free-radical reactions. Although in vivo H_2O_2 is always present at a low steady-state concentration, its reaction with DNA radicals will be negligible as compared to a reaction with O_2. However, such reactions may no longer be negligible in model studies using Fenton systems (Theruvathu et al. 2001) or in the deactivation of micro-organisms by H_2O_2 (Chap. 12.7).

6.8 Addition to C–C and C–N Double Bonds

6.8.1 Addition to C–C Double Bonds

The addition of carbon-centered radicals to C-C double bonds (for a review see Giese 1983) is the key reaction in the free-radical-induced polymerization. In general, the rate constants of these reactions are only moderately high, but this process becomes fast and efficient, because in technical applications the polymerizing olefin is usually present at high concentrations. In aqueous solutions, the rate constant of the addition of the hydroxyethyl radical to ethene [reaction (29)], a non-activated C-C double bond, has been determined at ~3×10^4 dm^3 mol^{-1} s^{-1} (Söylemez and von Sonntag 1980).

$$^{\bullet}CH_2\text{-}CH_2OH + CH_2{=}CH_2 \rightarrow HOCH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2^{\bullet} \quad (29)$$

Alkyl radicals are nucleophilic radicals (cf. Walbiner et al. 1995; Wu and Fischer 1995; Wu et al. 1995; Heberger and Lopata 1998), and the preferred position of addition at a polarized C-C double bond is reversed compared to that of the electrophilic $^{\bullet}OH$. Thus, in the case of Ura, the hydroxymethyl radical adds preferentially to the *C*(6)-position [$k \approx 10^4$ dm^3 mol^{-1} s^{-1} (Schuchmann et al. 1986; Chap. 10.5)].

6.8.2 Addition to C–N Double Bonds

Technically, the addition of carbon-centered radicals to C-N double bonds is as yet of little if any importance. In the free-radical chemistry of DNA it plays, however, a considerable role in the formation of the *C*(5′)-*C*(8) linkage between the sugar moiety and the purines (Chap. 10.5). Because of its importance, even an immune assay has been developed for the sensitive detection of this kind of damage in DNA (Chap. 13.2). The addition of the *C*(5′) radical to the *C*(8) position of a purine is obviously facilitated for steric reasons (formation of a six-membered ring), but the same kind of reaction also occurs as an intermolecular reaction. Since alkyl radicals are nucleophilic, the rate of this reaction is noticeably increased upon protonation of the purine (Aravindakumar et al. 1994; for rate constants see Chap. 10.5).

This addition reaction is not restricted to α-hydroxyalkyl radicals, although this type of radical has been most widely investigated. Thus, allylic radical derived from 5MeCyt (Zhang and Wang 2003) and radicals derived from amino acids (Elad and Rosenthal 1969) are also reported to undergo this reaction. In DNA, they play a role in the formation of tandem lesions (Chap. 12.5), and it is likely that this kind of reaction contributes to free-radical-induced DNA/DNA and DNA/protein cross-linking.

6.9 β-Fragmentation Reactions

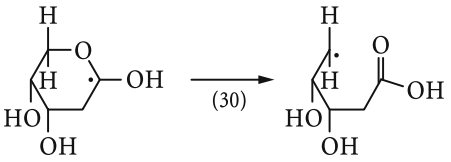
6.9.1 Homolytic Fragmentation

Carbon-centered radicals may undergo homolytic β-fragmentation reactions, whereby an olefin and a new radical is formed. This reaction is, in fact, the reverse of the polymerization reaction. With neighboring C-C bonds, these β-fragmentation reactions are usually slow, and only observable, at least on the pulse radiolysis time-scale with negatively-charged polymeric radicals whose lifetime is prolonged by electrostatic repulsion. Then, even the situation of equilibrium polymerization may be approached (Ulanski et al. 2000; Chap. 9.4).

In the nucleobases, this type of reaction is not possible due to the lack of adequate structural elements.

There is a wealth of information available on the free-radical reactions of carbohydrates in aqueous solution and in the solid state (for reviews see von Sonntag 1980; von Sonntag and Schuchmann 2001). According to this, there is no indication that β-fragmentation involving C-C double bond formation is a major process in such systems. However, β-fragmentation forming a C-O double bond is quite common, e.g., in disaccharides, where such a process can lead to the scission of the glycosidic linkage. It is also observed in monosaccharides, notably in 2-deoxyribose in the crystalline state (Hüttermann and Müller 1969a,b; von Sonntag et al. 1974; Schuchmann et al. 1981), where it is present as the β-py-

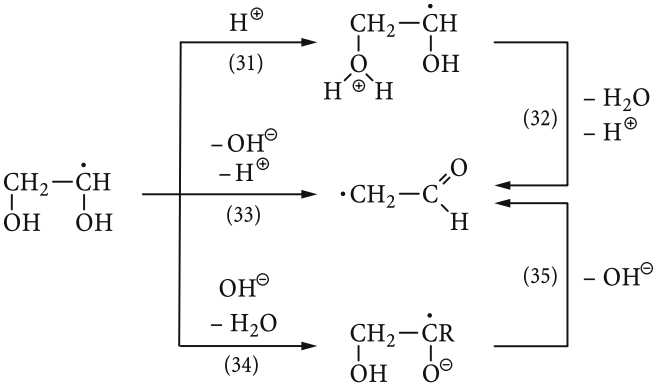
ranose form. Reaction (30) is one step in an interesting and very efficient chain reaction which is governed by the crystal structure (Schuchmann et al. 1981).



When the binding energy of the bond to be broken is considerably lower than that of a C-C single bond such as a C-SR bond, the rate of β-fragmentation can go up dramatically. This is the reason why the addition of thiyl radicals to the double bonds of the nucleobases is so strongly reversible (Chaps 7.4 and 10.5).

6.9.2 Water Elimination, Heterolytic β-Fragmentation and Formation of Radical Cations

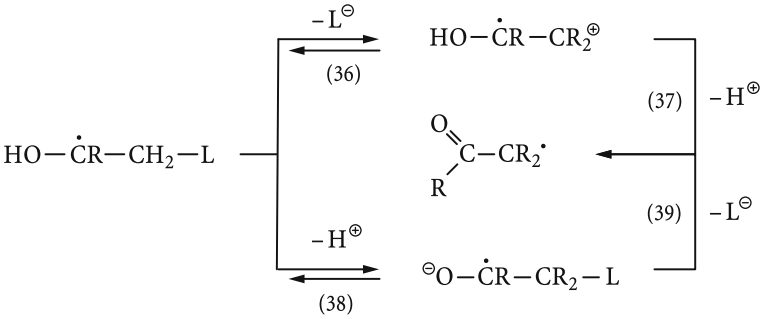
In DNA, the carbohydrate part has no free OH groups. Yet, salient aspects have been obtained by studying the free-radical chemistry of carbohydrates (von Sonntag 1984). A general feature in carbohydrate free-radical chemistry is the water elimination of 1,2-dihydroxyalkyl radicals [reactions (31)-(35)].



This reaction has been first observed by EPR (Buley et al. 1966) and subsequently established by product studies on ethylene glycol (Seidler and von Sonntag 1969; von Sonntag and Thoms 1970; Burchill and Perron 1971), meso-erythritol (Dizdaroglu et al. 1972) and 2-deoxy-D-ribose (Hartmann et al. 1970; Herak and Behrens 1986), D-ribose (von Sonntag and Dizdaroglu 1977; Herak and Behrens 1986) and D-glucose (Dizdaroglu et al. 1975b); for reviews on carbohydrate free-radical chemistry see von Sonntag (1980); Ershov (1998); von Sonntag and Schuchmann (2001); for a review on the photochemistry of carbohydrates see Binkley (1981). The formylmethyl radical that is formed by water elimination from ethylene glycol [reactions (31)-(35)] is capable of abstracting a hydrogen

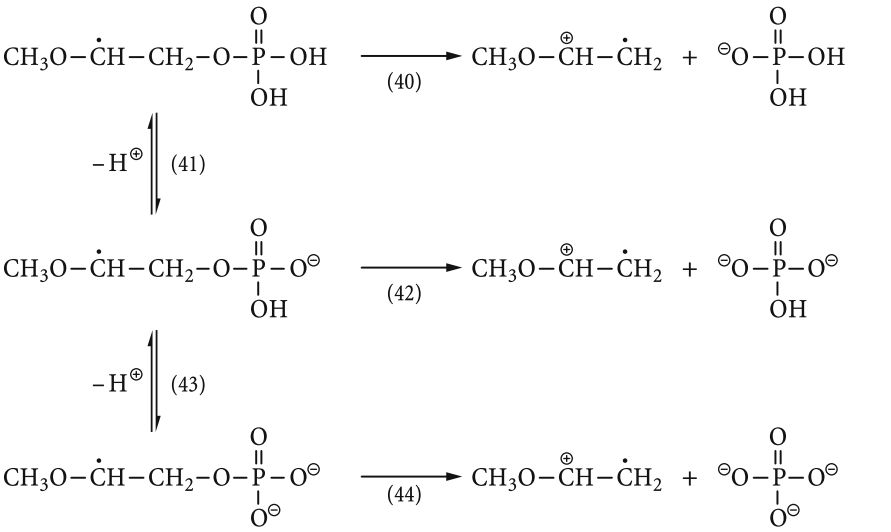
from the substrate (von Sonntag and Thoms 1970). Possibly, this type of radical has to be made responsible for the formation of DNA SSBs induced by glycerol-derived radicals at high scavenger concentrations (Chap. 12.1).

The mechanism of this water elimination and related reactions, where the OH group in β-position is replaced by another leaving group (L), has been studied in some detail (Samuni and Neta 1973; Steenken et al. 1974; Behrens and Schulte-Frohlinde 1976; Behrens and Koltzenburg 1985; Schuchmann et al. 1995; Müller et al. 1997; Bales et al. 2001). Two processes which compete with one another have been recognized: heterolytic cleavage of the C-L bond which results in the formation of a radical cation followed by proton loss [reactions (36) and (37)] and the dissociation of the hydroxyl group followed by the loss of the leaving group [reactions (38 and (39)].



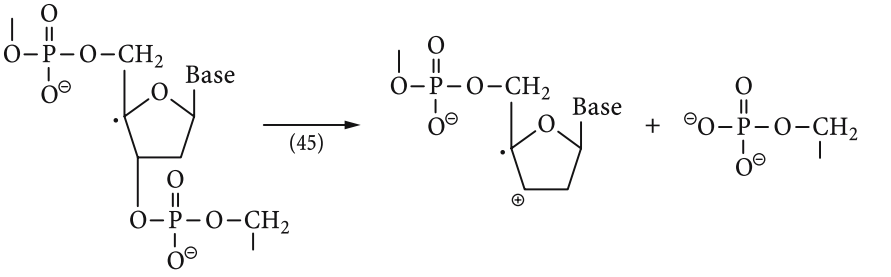
The rate of the elimination of the ligand [reactions (39) and (39)] is determined by the electrophilicity of the frame and the nucleofugacity of the ligand (Behrens and Koltzenburg 1985). Deprotonation of the frame [reaction (38)] strongly reduces its electrophilicity, and thus the radical anion may eliminate quite well also ligands which have an intrinsic poor nucleofugacity such as OH [see below; cf. reaction (39)]. The rate of reaction (38) is determined by the pK_a value of the α-hydroxyalkyl radical and is thus strongly influenced by the substituents R and L. As a measure of the nucleofugacity the pK_a value of the conjugate acid of the leaving group may be taken: HCl (negative pK_a), CH_3CO_2H (4.7), NH_4^+ (9.3), i.e. a high pK_a corresponds to a low nucleofugacity. Thus, the OH group (pK_a(H_2O) = 14) is also a poor leaving group. However, upon protonation it becomes a very good leaving group (pK_a(H_3O^+) is negative), and this is the reason why the water elimination is also acid catalyzed [cf. reaction (31); Steenken 1979; Steenken et al. 1986a].

A much simpler situation is the anion loss from an α-alkoxyalkyl radical with a leaving group in the β-position, such as the phosphate release from the 2-phosphato-methoxyethyl radical (Behrens et al. 1978; for some further reactions of β-(phosphatoxy)alkyl radicals see Whitted et al. 1999; Crich et al. 2000). This reaction only proceeds by a heterolytic cleavage into a radical cation and a phosphate ion. The rate of this reaction strongly depends on the protonation state of the phosphate group [reactions (40)–(44)].



From the viewpoint of the phosphate group, release of a proton or a radical cation is governed by the same principle. With increasing deprotonation (i.e., increasing negative charge on the phosphate group), the rate of the release of a positively charged species (proton or radical cation) slows down. As a consequence, there is a good relationship of the rate of deprotonation of H_3PO_4, $H_2PO_4^-$ and HPO_4^{2-} (as can be calculated, see above, from their pK_a values at 2.1, 7.2 and 12.7) and the rates of phosphate release from the 2-phosphato-methoxyethyl radical at different protonation states (Table 6.6). There is also a good correlation between the rate of phosphate elimination and some EPR coupling constants of the eliminating radical which indicates that considerable polarization exists already in the radical (Behrens et al. 1978).

The same type of reaction, starting from the radical at *C*(4′), leads to strand breakage in DNA [reaction (45) (Dizdaroglu et al. 1975a); Chap. 12.4].

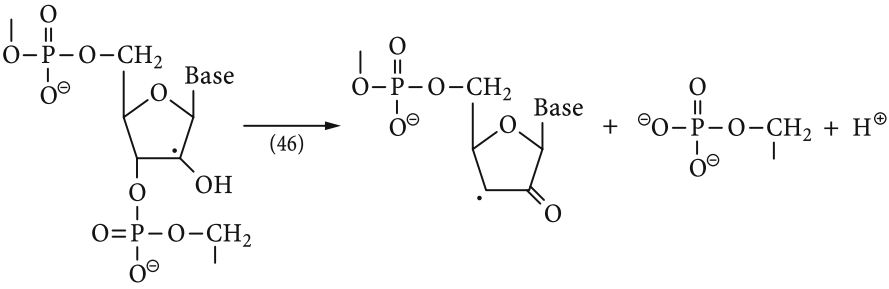


The release of phosphate from the α-hydroxy-β-phosphato radicals derived from glycerol phosphates (Samuni and Neta 1973; Steenken et al. 1974) is much faster [$k > 10^5$ s^{-1} (Schuchmann et al. 1995)] than that of the corresponding methoxy-substituted radical (k = 0.1-1 s^{-1}, Table 6.6). Although methyl-substitution at the oxygen will increase the electrophilicity of the frame, this effect seems not to be

Table 6.6. Approximate rate constants of phosphate release from some α-methoxy-β-phosphatoalkyl radicals. (Behrens et al. 1978)

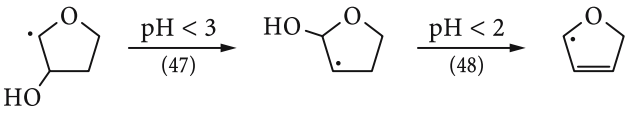
Radical	Rate constant/s^{-1}
$^{\bullet}CH(OCH_3)CH_2OPO_3H_2$	10^6
$^{\bullet}CH(OCH_3)CH_2OPO_3H^-$	10^3
$^{\bullet}CH(OCH_3)CH_2OPO_3^{2-}$	0.1 - 1
$^{\bullet}CH(OCH_3)CH_2OPO_3(CH_2CH_2OCH_3)H$	10^7
$^{\bullet}CH(OCH_3)CH_2OPO_3(CH_2CH_2OCH_3)^-$	10^3 - 10^4
$^{\bullet}CH(OCH_3)CH_2OPO_3(CH_2CH_2OCH_3)_2$	4×10^7

sufficient to explain these differences in rate. It may hence be envisaged that a concerted proton loss speeds-up the phosphate release in the case of the α-hydroxy-β-phosphato radicals. In addition, intramolecular deprotonation by the phosphate group could further enhance the rate. In the free-radical chemistry of polyribonucleotides and RNA, this must have a bearing on the rate of strand breakage starting from the *C*(2′) radical [reaction (46); Chap. 11.2].



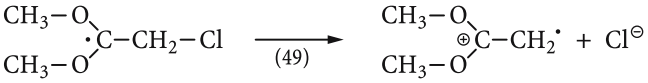
The radical cations formed in all these reactions are not stable but react quite rapidly with water. This reaction is to a large extent kinetically controlled, and hence also radicals that are thermodynamically disfavored are formed as well. Table 6.7 shows a compilation of the ratios of radicals formed as studied by EPR spectroscopy.

Proton catalysis which regenerates the radical cation ultimately leads to the thermodynamically most stable radical. This can even proceed in two well-separated steps [reactions (47) and (48)]. Here, the second step is much slower and hence only observed at lower pH (Behrens et al. 1982).

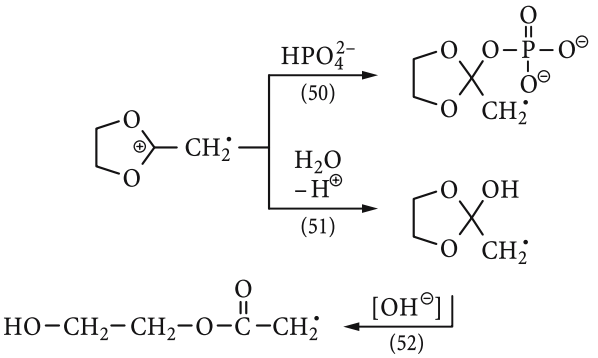


The gain in stabilization by substituents is compared for radicals and cations in Table 6.8. For radicals the change from methyl to ethyl or even tertiary butyl is not linked to a large gain in stabilization energy. In cations, however, alkyl and alkoxyl substituents have a dramatic effect. Thus, the stabilizing effect of substituents on radical cations is mainly due to the stabilization of the cation and only to a small extent to that of the radical.

Considerable stabilization is achieved by two methoxy groups, and the radical cation resulting from reaction (49) is stable with respect to its reaction with water ($k < 10^3\ s^{-1}$) (Behrens et al. 1980). Its radical properties are not altered by the stabilization of the cation, and hence these radical cations decay bimolecularly at diffusion-control rates.



The methylenedioxolane radical cation is somewhat less stable and reacts with water at a rate of $7 \times 10^{-2}\ s^{-1}$ [reaction (51)] and (as other radicals cations also do) with phosphate ions [reaction (50)]. The radical formed in reaction (51) opens the ring upon deprotonation [reaction (52)]. Reactions analogous to reaction (61) can be used as a probe to trap short-lived radical cations.



As expected, other radicals with a good leaving group in β-position can give rise to the formation of radical cations (Koltzenburg et al. 1982), but it is quite surprising that radical cations can also be formed under certain conditions when the leaving group is in the γ-position, for example, reaction (53) (Koltzenburg et al. 1983). The lifetime of the radical is ca. 10^{-5} s and that of its radical cation with respect to its reaction with water has been estimated at about 10^{-8} s [using the competition with phosphate; cf. reaction (50)].

Table 6.7. Hydrolysis of radicals with good leaving groups (L) in β-position to the radical center. (Behrens et al. 1982)

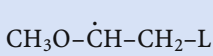
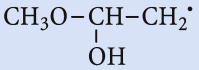
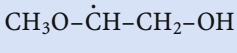
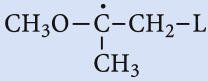
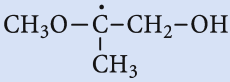
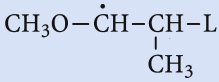
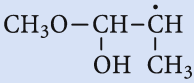
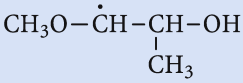
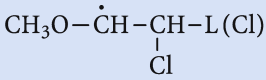
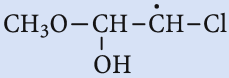
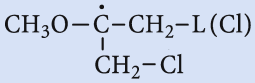
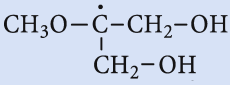
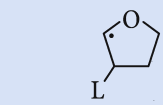
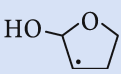
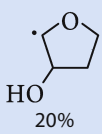
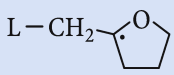
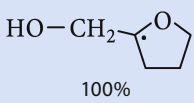
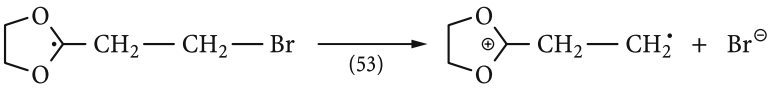
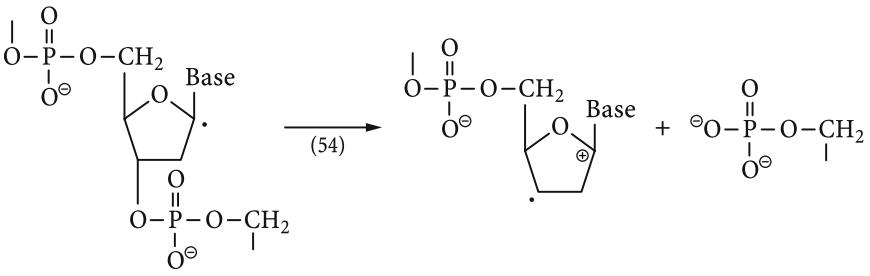
Radical	Product radicals	
	 70%	 30%
	 100%	
	 70%	 30%
	 100%	
	 100%	
	 80%	 20%
	 100%	

Table 6.8. Stabilization energies of some cations and radicals relative to the corresponding methyl species. (According to G. Koltzenburg, compiled in von Sonntag 1987)

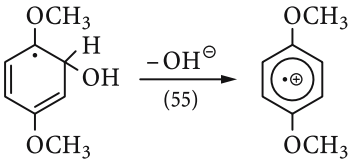
Cation	kJ mol^{-1}	Radical	kJ mol^{-1}
$^{+}CH_3$	0	$^{\bullet}CH_3$	0
$^{+}CH_2CH_3$	630	$^{\bullet}CH_2CH_3$	71
$^{+}CH_2CH_2CH_3$	685		
$^{+}CH_2CH(CH_3)_2$	825		
$^{+}CH(CH_3)_2$	965	$^{\bullet}CH(CH_3)_2$	122
$^{+}C(CH_3)_3$	1195	$^{\bullet}C(CH_3)_3$	193
$^{+}CH_2OCH_3$	1160		
$^{+}CH(OCH_3)_2CH_3$	1350		
$^{+}CH(OCH_3)_2$	1490		
$^{+}C(OCH_3)_3$	1580		



The observation of this γ-elimination reaction has raised the question, whether in DNA the related *C*(1′) radical can also lead to strand breakage. However due to the negative charge at the phosphate group in 3′-position, the rate of the γ-elimination reaction (54) should be considerably slower than that of reaction (53).



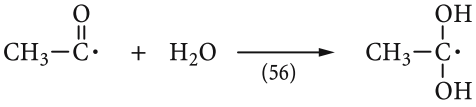
Aromatic radical cations are readily formed and have a measurable lifetime when they are stabilized by electron-donating substituents [e.g., reaction (55); O'Neill et al. 1975].



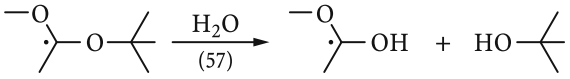
They may be produced by biphotonic excitation or by a strong oxidant such as $SO_4^{\bullet-}$. The radical cations of methyl-substituted benzenes react with water forming mainly hydroxycyclohexadienyl radicals and deprotonate at methyl only to a small extent yielding benzyl-type radicals (Sehested and Holcman 1978; Russo-Caia and Steenken 2002). As expected, the lifetime of these radical cations increases with increasing methyl substitution (toluene: $k > 5 \times 10^7\ s^{-1}$, *p*-xylene: $k \approx 2 \times 10^6\ s^{-1}$, 1,2,4,5-tetramethylbenzene: $k \approx 4 \times 10^4\ s^{-1}$). In strongly acidic solution, the reaction becomes reversible, and aromatic radical cations can also be generated via $^{\bullet}OH$ under such conditions. Since the deprotonation at methyl is irreversible, benzyl-type radicals are the final products in acid solutions (Sehested et al. 1975, 2002). Aromatic radical cations also undergo oxidation of substituents in the side chain (Wang et al. 1993; Baciocchi et al. 1996, 1997, 1998, 1999).

6.10 Hydration and Hydrolysis of Radicals

In principal, all steps in these water-elimination reactions are reversible. Because in the reactions discussed above the final products are thermodynamically more stable, the equilibrium lies fully on the right side (i.e., the reverse rates are very slow). However, there are also cases where hydration of a radical is a fast process. For example, reaction (67) occurs at a rate of $2 \times 10^4\ s^{-1}$ (Schuchmann MN and von Sonntag 1988), i.e., a million times faster than the rate of hydration of its non-radical parent, acetaldehyde.



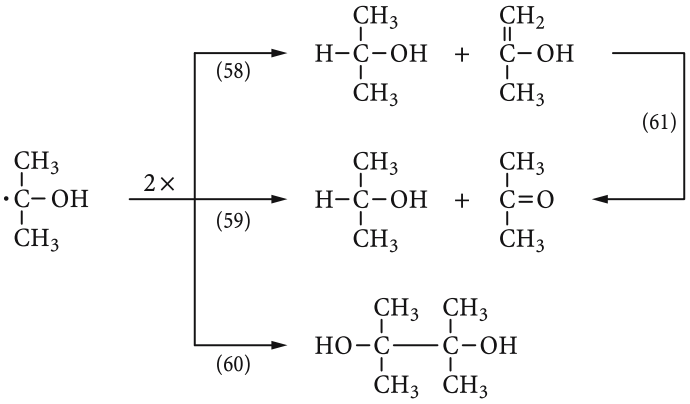
The hydrolysis of the glycosidic linkage in disaccharides is usually a slow and acid-catalyzed process. It is remarkably speeded up, when at the site of the glycosidic linkage the hydrogen has been replaced by a lone electron [reaction (68)] (for reviews see von Sonntag 1980; von Sonntag and Schuchmann 2001).



Details have not been investigated. Whether in DNA such a reaction plays also a role in the elimination of the nucleobases from the *C*(1′) radical, is as yet not known.

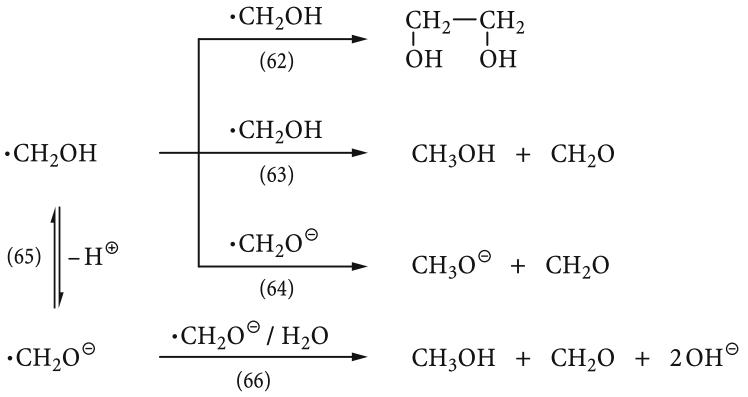
6.11 Bimolecular Termination Reactions

In general, carbon-centered radicals disappear by recombination and disproportionation (when β-hydrogens are available). The rate of reaction is usually somewhat below diffusion-controlled and may require some activation energy (e.g., ~19 kJ mol^{-1} for $^{\bullet}C(CH_3)_2OH$; Mezyk and Madden 1999). Although recombination is usually the preferred reaction, disproportionation plays an increasing role, when the number of hydrogens in β-position is increased. A case in point is the series of α-hydroxyalkyl radicals. With the hydroxymethyl radical, where only one hydrogen (that at oxygen) is available for the disproportionation reaction, the disproportionation/recombination ratio is 0.17 (Wang et al. 1996) [cf. reactions (62) and (63)]; this ratio increases to ~2.3 in the case of the α-hydroxyethyl radical (Seddon and Allen 1967; four hydrogens available for disproportionation) and with the 2-hydroxyprop-2-yl this ratio is about 10 (Lehni and Fischer 1983). In the latter case seven hydrogens, six at the methyl groups and one oxygen-on, now facilitate disproportionation which is now the preferred mode of radical termination.. Although the formation of acetone [reaction (59)] is thermodynamically much favored over the formation of its enol [reaction (58)], the latter reaction is kinetically chosen preferentially (von Sonntag 1969; Blank et al. 1975; see also Laroff and Fischer 1973; Bargon and Seifert 1974).



The hydroxymethyl radicals display an interesting feature. Their neutral forms largely dimerize [reaction (62)], but their radical anions disproportionate [reaction (66)], although there are no longer any hydrogens available for disproportionation (Wang et al. 1996). It has been suggested that this surprising disproportionation reaction may be due to a head-to-tail combination of the hydroxymethyl radical anions or it may proceed by a water-assisted reaction.

In termination reactions, all mesomeric structures may contribute. Cases in point, where one would not immediately expect this to play a significant role are the α-carboxymethyl radicals (Wang et al. 2001). For some of the nucleobase radicals also more than one mesomeric structure may be written (Chap. 10), and it is not unlikely that also here this aspect has to be taken into account.



The rates of these bimolecular termination reactions are usually close to diffusion controlled except for equally charged radicals (Ulanski et al. 1997), notably polymeric radicals (Chap. 9.3). DNA belongs to this group.

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Heteroatom-Centered Radicals

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7.1 General Remarks

With the nucleobases, radicals may be formed which carry the spin predominantly at a heteroatom, oxygen or nitrogen. In addition, the importance of glutathione and possibly also the surrounding proteins in modifying DNA free-radical damage in a cellular environment will at one stage involve heteroatom-centered radicals, that is, also at sulfur. In many aspects, heteroatom free-radical chemistry differs from that of carbon-centered radicals, although these radicals are often highly delocalized with a considerable spin density at carbon.

When the binding energy of a hydrogen to a heteroatom is weak, heteroatom-centered radicals are readily produced by H-abstraction or one-electron oxidation followed by H^+ loss. Typical examples are phenols (e.g., vitamin E in non-aqueous media), tryptophan and related compounds and thiols. Deprotonation of radical cations is indeed often a source of heteroatom-centered radicals even if a deprotonation at carbon or OH^- addition upon reaction with water would be thermodynamically favored. The reason for this is the ready deprotonation at a heteroatom (Chap. 6.2).

Among the oxygen-centered radicals, $^{\bullet}OH$ and $ROO^{\bullet}$ play a major role, and their chemistries are discussed in Chapters 3 and 8, respectively. Here we deal with some other oxygen-centered species such as $RO^{\bullet}$ and phenoxyl radicals. Oxyl radicals are formed in the bimolecular decay of peroxyl radicals (Chap. 8.8), thermal and photolytic decomposition of peroxides or their reductive cleavage (Chaps 2.4 and 3.3). Due to their short lifetime, which is discussed below in some detail, their DNA damaging property is only marginal (Chap. 2.4). The aromatic phenoxyl radicals, on the other hand, are of little reactivity, but they seem to take part in DNA-protein cross-linking. They have also some oxidizing properties and may be taken as model systems for the guanine radical, $G^{\bullet}$, whose chemistry is as yet not fully elucidated.

The chemistries of nitrogen-centered and sulfur-centered radicals have been reviewed in detail (Alfassi 1997, 1999), and here only some aspects can be discussed that seem pertinent to the formation, reactions and repair of DNA radicals.

A common feature of heteroatom-centered radicals is that they react reversibly, only slowly or not at all with O_2 (Schuchmann and von Sonntag 1997), and this property is shared by the purine radicals $G^{\bullet}$ and $A^{\bullet}$ (Chap. 10.2).

7.2 Oxygen-Centered Radicals

The logarithm of the rate constants of H-abstraction by reactive oxygen species decreases with decreasing O–H bond dissociation energy from $^{\bullet}OH$ [BDE(HO–H) = 497 kJ mol^{-1}] > $RO^{\bullet}$ [BDE(RO–H) = 439 kJ mol^{-1}] > $ROO^{\bullet}$ [BDE(ROO–H) = 372 kJ mol^{-1}], and this sequence also includes the even less reactive metal-oxo complexes, e.g., permanganate (Mayer 1998).

7.2.1
β-Fragmentation, 1,2-H-Shift and H-Abstraction Reactions

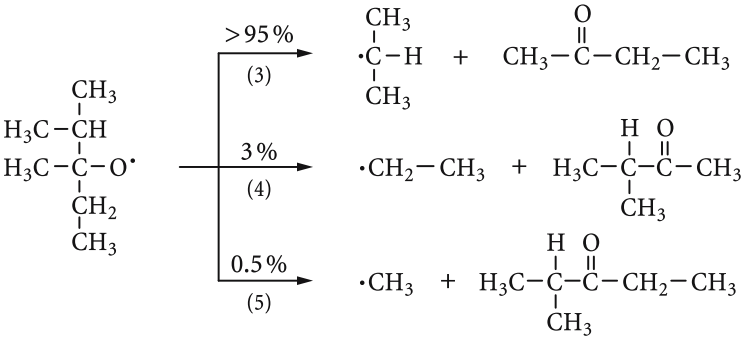
Many *O*-centered radicals undergo facile β-fragmentation. For example, acyloxyl radicals which are intermediates in the electrolytic oxidation of acids (Kolbe electrolysis), rapidly decompose into alkyl radicals and CO_2 [reaction (1)]. The rate of these reactions is in the order of 10^9 s^{-1} and increases with increasing branching of the alkyl substituent, i.e., decreasing C-$CO_2^{\bullet}$ bond energy (Table 7.1).

$$R\text{-}C(O)O^{\bullet} \rightarrow R^{\bullet} + CO_2 \qquad (1)$$

Similarly, the β-fragmentation of tertiary alkoxyl radicals [reaction (2)] is a well-known process. Interestingly, this unimolecular decay is speeded up in a polar environment. For example, the decay of the *tert*-butoxyl radical into acetone and a methyl radical proceeds in the gas phase at a rate of 10^3 s^{-1} (for kinetic details and quantum-mechanical calculations; see Fittschen et al. 2000), increases with increasing solvent polarity (Walling and Wagner 1964), and in water it is faster than 10^6 s^{-1} (Gilbert et al. 1981; Table 7.2).

$$R_3CO^{\bullet} \rightarrow R^{\bullet} + R_2C{=}O \qquad (2)$$

When different substituents can be cleaved by β-scission the larger substituent leaves preferentially [Rüchardt 1987; cf. reactions (3)-(5)].



When alkoxyl radicals contain a hydrogen atom at the neighboring carbon, a rapid 1,2-H-shift ($k \approx 10^6$ s^{-1} or even faster) occurs in aqueous solution [reaction (6); Berdnikov et al. 1972; Gilbert et al. 1976, 1977; Schuchmann and von Sonntag 1981] with a KIE ≈ 50 in the $CH_3CH_2O^{\bullet}/CD_3CD_2O^{\bullet}$ system (Bonifacic et al. 2003).

$$HR_2CO^{\bullet} \rightarrow {\bullet}C(OH)R_2 + R_2C{=}O \qquad (6)$$

This process must be water assisted (see also Elford and Roberts 1996), since this reaction requires a high activation energy in the gas phase (Batt et al. 1981;

Table 7.1. Rate of decay of some acyloxyl radicals. (Hilborn and Pincock 1991)

Substituent	$k / 10^9\ s^{-1}$
CH_3	<1.3
CH_2CH_3	2.0
$(CH_3)_2CH$	6.5
$(CH_3)_3C$	11

Table 7.2. Activation parameters and rate constants (at 295 K) of the β-scission of the *tert*-butoxyl radical

Solvent	$\log(A/s^{-1})$	E_a/kJ mol^{-1}	$k/10^3\ s^{-1}$	Reference
Gas phase	14.04	62.5	1.63	Batt et al. (1998)
CCl_4	13.4±0.5	53±4	10±2	Tsentalovich et al. (1998)
C_6H_6	13.4±0.6	52±4	14±3	Tsentalovich et al. (1998)
CH_3CN	13.2±0.4	48±3	64±11	Tsentalovich et al. (1998)
$(CH_3)_2COH$	12.4±0.6	40±5	190±40	Tsentalovich et al. (1998)
CH_3CO_2H	12.1±0.3	37±3	340±60	Tsentalovich et al. (1998)
H_2O			1400±150	Erben-Russ et al. (1987)

Adams et al. 1982; Saebo et al. 1983; Sosa and Schlegel 1987). The resulting hydroxyalkyl radical is of lower energy, and this is the driving force of this reaction (for the opposite situation in sulfur free-radical chemistry see below). In secondary alkoxyl radicals the 1,2-H-shift reaction may be even faster than the also very rapid β-fragmentation. For example, this ratio is near 3-4 in the case of $CH_3CH(O^{\bullet})OCH_2CH_3$ while the corresponding oxyl radical derived from poly(ethylene glycol) only undergoes β-fragmentation (Schuchmann and von Sonntag 1982; cf. also Gröllmann and Schnabel 1980). In the poly(ethylene glycol) system, β-fragmentation is speeded up due to the formation a stabilized $-OCH_2^{\bullet}$ radical, while in the former case a less stabilized $^{\bullet}CH_3$ radical has to be eliminated.

Similar to $^{\bullet}OH$, alkoxyl radicals are also good H-abstractors [e.g., reaction (7)], although they react with much lower rates (e.g., $k_7 = 2.6 \times 10^5\ dm^3\ mol^{-1}\ s^{-1}$; Ellison et al. 1972; the tertiary butoxyl has a similar rate constant; Paul et

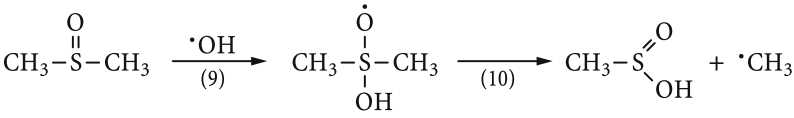
al. 1978; for kinetic isotope effects in H-abstraction reactions, see Tanko et al. 2001).

$$CH_3O\bullet + CH_3OH \rightarrow CH_3OH + \bullet CH_2OH \quad (7)$$

Alkoxyl radicals are practically as readily reduced by Fe^{2+} as $^{\bullet}OH$ (e.g., reaction (8), $k = 3 \times 10^8$ dm^3 mol^{-1} s^{-1}; Mihaljevic et al. 1999). Due to the high rate of competing reactions (see above), such a reaction can only be observed at high Fe^{2+} concentrations.

$$(CH_3)_3CO^{\bullet} + Fe^{2+} + H^+ \rightarrow (CH_3)_3COH + Fe^{3+} \quad (8)$$

Oxyl radicals are also formed upon the addition of $^{\bullet}OH$ to sulfoxides. Like other tertiary alkoxyl radicals they undergo rapid fragmentation [reactions (9) and (10); Veltwisch et al. 1980].

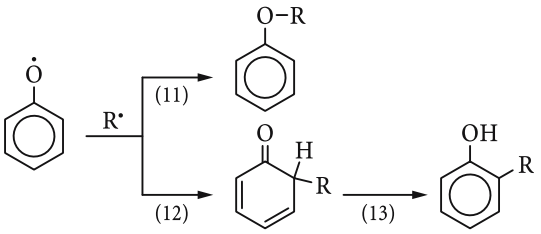


The tertiary butoxyl radical reacts with sulfides by H-abstraction (Adams 1970) rather than by an oxidation of the sulfur as •OH does (see below).

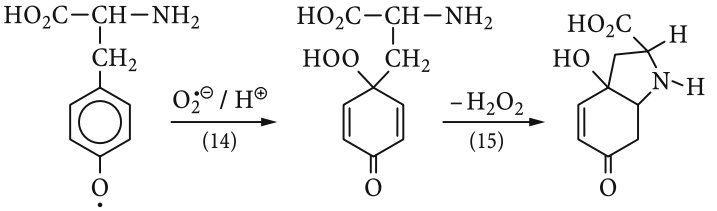
Because of the fast rates of [reactions (2) and (6)] in aqueous solutions, reactions of alkoxyl radicals with DNA components can be neglected unless created within the DNA or in its very neighborhood (Chap. 2.4).

7.2.2 Phenoxyl Radicals

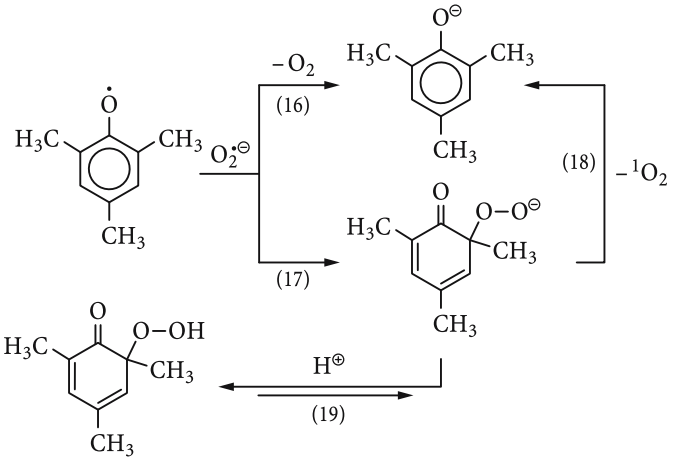
Phenoxyl radicals have considerable spin density at the carbons in *ortho*- and *para*-position. Yet like G$^{\bullet}$, they do not react with O_2 (Jin et al. 1993), unless electron-donating groups provide a high enough electron density (Wang et al. 1994). In this case, O_2 must bind at carbon because a reaction at oxygen would yield a very unstable trioxyl radical. In their cross-termination reactions, phenoxyl radicals do not combine at oxygen yielding the corresponding peroxide (the reaction is endothermic, G. Merényi, pers. comm.). They react, however, at oxygen by forming C-O linkages [reaction (11); Benn et al. 1979; Ye and Schuler 1989, and DNA-protein cross-linking may indeed occur via such a reaction; Margolis et al. 1988; Dizdaroglu et al. 1989; Gajewski and Dizdaroglu 1990]. Upon addition at carbon in the *ortho* and *para* positions [e.g., reaction (12)], a cyclohexadienone is formed which rapidly reverts to the corresponding phenol [reaction (13); Bausch et al. 1976; Benn et al. 1979; for the rate constant see Capponi et al. 1986]. With the prototype, various dimers are formed (Ye and Schuler 1989). In the case of tyrosine-derived phenoxyl radical, the major one is 2,2′-bityrosyl (Jin et al. 1993).



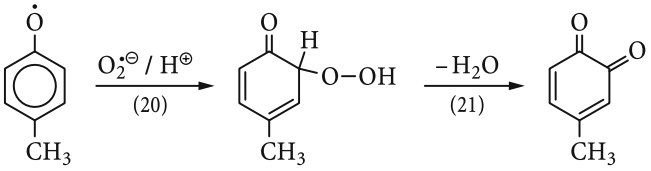
Phenoxyl radicals are oxidizing radicals (for a compilation of redox potentials see Wardman 1989). Thus, in their reactions with $O_2^{\bullet-}$ (E^7 = -0.3 V) there is ample driving force for a reduction by ET [cf. reaction (16)], and this has been thought for a long time to be the only (Hunter et al. 1989) or at least a major process, depending on the reduction potential of the (substituted) phenoxyl radical (Jonsson et al. 1993). Yet in the tyrosine system, despite of the high reduction potential of tyrosine phenoxyl radical (E^7 = 0.64 V), the by far dominating process is addition, and the intermediate adduct is locked in by a Mannich reaction [reactions (14) and (15); Jin et al. 1993].



Reduction of a phenoxyl radical by $O_2^{\bullet-}$ can nevertheless occur by an addition/elimination sequence as shown in reactions (17) and (18) (d'Alessandro et al. 2000). In the given case, the pK_a value of the hydroperoxide is 11.3 [equilibrium (19)], and O_2 elimination occurs only from the anion [reaction (18); $k = 1.3 \times 10^{-5}$ s^{-1} at 23 °C, E_a = 105 kJ mol^{-1}].



In addition to the O_2 elimination reaction (17) and the Mannich reaction (14), phenoxyl/$O_2^{\bullet-}$ combination products can undergo a water elimination if the position where $O_2^{\bullet-}$ has added to carries an H atom [e.g., reactions (20 and (21); d'Alessandro et al. 2000].



As mentioned above, these reactions are potentially of some heuristic importance also for DNA in so far as little is known about the fate of G$^{\bullet}$ (notably its bimolecular decay; Chap 10.2). Both G$^{\bullet}$ and phenoxyl radicals share quite some properties; they are aromatic with high spin density at oxygen, reasonably strong oxidants, do not react with O_2 at an appreciable rate, but quite readily with $O_2^{\bullet-}$.

Many antioxidants quoted as potential protective agents against free-radical-induced DNA damage have more than one phenolic group. Their chemistry is, therefore, also of some interest in the present context. The semiquinone radicals, derived from hydroquinone by one-electron oxidation or from 1,4-benzoquinone by one-electron reduction, are in equilibrium with their parents (Roginsky et al. 1999), and these equilibria play a role in the autoxidation of hydroquinone (Eyer 1991; Roginsky and Barsukova 2000). Superoxide radials are intermediates in these reactions.

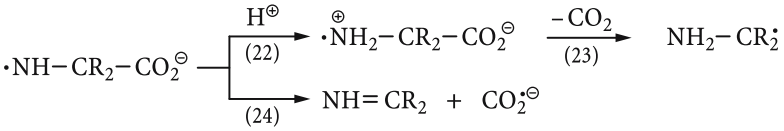
7.3 Nitrogen-Centered Radicals

In DNA free-radical chemistry, *N*-centered radicals are generated from some nucleobases upon one-electron oxidation (followed by H^+ loss). They are also considered as important intermediates in the purine free-radical chemistry. It is, therefore, worthwhile to address very briefly some of the chemistry of *N*-centered radicals that were encountered in amines and amino acids.

The reaction of $^{\bullet}$OH with trimethylamine in aqueous solution leads to the formation of the alkylaminium radical cation [$CH_3N^{\bullet+}$], aminoalkyl radicals [$(CH_3)_2NCH_2^{\bullet}$] and protonated aminoalkyl radicals [$(CH_3)_2N(H^+)CH_2^{\bullet}$] which have markedly different properties (Das and von Sonntag 1986; for a theoretical study see Armstrong et al. 1992). The aminoalkyl radicals react rapidly with O_2 thereby giving rise to $O_2^{\bullet-}$ (Das et al. 1987).

Aminyl radicals formed in the reaction of $^{\bullet}$OH with amino acids ($^{\bullet}$NH-CR_2-CO_2^-) are oxidizing radicals and readily react with phenols (Bonifacic et al. 2000a). In the presence of a proton donor, they are converted into the corresponding radical cation [reaction (22)] which immediately decarboxylates [reaction (23); see also Lü et al. (2001)]. It also may undergo β-fragmentation thereby forming $CO_2^{\bullet-}$ [reaction (24); Bonifacic et al. 2000a; Hug et al. 2000a; Stefanic

et al. 2001; for further studies on the free-radical-induced decarboxylation of amino acids see Mönig et al. 1985].



The formation of the reducing aminoalkyl radical formed in reaction (23) that had escaped detection in the earlier studies (Neta and Fessenden 1971) has now also been confirmed by EPR (Hug et al. 2000b).

As purine free-radical chemistry is concerned, the 1,2-H-shift reaction (25) is of special interest.

$$^{\bullet}\mathrm{NR\text{-}CHR_2} \rightarrow \mathrm{HNR\text{-}{}^{\bullet}CR_2} \qquad (25)$$

The rate of such an (exothermic) reaction is not yet known. In the amino acids, β-fragmentation [reactions (22)–(24)] compete successfully (Bonifacic et al. 2000a; for DFT calculations see therein).

Tryptophan and its derivatives such as the *Hoechst* compounds (Adhikary et al. 2000) have reduction potentials below that of $G^{\bullet}$ (tryptophan: $E^7 = 1.0$ V; Jovanovic and Simic 1985) and thus are capable of repairing some of the DNA damage (for a review on indol free-radical chemistry see Candeias 1998; for the thermochemistry of *N*-centered radicals see Armstrong 1998). In these reactions, radical cations and *N*-centered radicals are formed. Similar to phenoxyl radicals, these radical react with $O_2^{\bullet-}$ mainly by addition despite the large difference in the redox potential which would allow an ET as well (Fang et al. 1998).

7.4 Sulfur-Centered Radicals

The chemistry of *S*-centered radicals is so different from that of *C*-centered radicals and so surprisingly varied that it has found considerable attention (for reviews besides the one mentioned above see von Sonntag and Schuchmann 1980a,b; Anklam and Margaretha 1989; Asmus 1990a,b; Armstrong 1990; for the thermochemistry of sulfur radicals see Griller et al. 1990; Armstrong 1999; for their structures see Armstrong and Chipman 1999; for thiyl radicals in biology see Wardman 1999). In the present context of free-radical-induced DNA damage, there is a special interest, because thiols, notably glutathione which is present in cells at rather high concentrations, achieves a reduction of free-radical-induced DNA damage caused by ionizing radiation in cellular systems (Alper 1979; for more recent reviews, see Quintiliani 1983; von Sonntag and Schuchmann 1990; Wardman and von Sonntag 1995; see also Chap. 12.11).

Table 7.3. Rate constants (in units of $dm^3\ mol^{-1}\ s^{-1}$) of the reaction of some radicals with thiols

Radical	Thiol	pH	Rate constant	Reference
$^{\bullet}CH_3$	Dithiothreitol	7	7.4×10^7	Reid et al. (2002)
$^{\bullet}CH_2OH$	2-Mercaptoethanol	10	1.3×10^8	Karmann et al. (1969)
	Dithiothreitol	7	6.8×10^7	Redpath (1973)
	Cysteamine	7.6	4.7×10^7	Adams et al. (1968)
$^{\bullet}C(CH_3)HOH$	2-Mercaptoethanol	10	2.3×10^8	Karmann et al. (1969)
	Dithiothreitol	7	9.6×10^7	
	Cysteamine	7.6	1.4×10^8	Adams et al. (1968)
$^{\bullet}C(CH_3)_2OH$	2-Mercaptoethanol	10	5.1×10^8	Karmann et al. (1969)
	Dithiothreitol	7	2.0×10^8	
$^{\bullet}CH_2C(CH_3)_2OH$	2-Mercaptoethanol	10	8.2×10^7	Karmann et al. (1969)
	Dithiothreitol	7	6.8×10^7	Redpath (1973)
	Cysteamine	7.6	1.8×10^7	Adams et al. (1968)
$^{\bullet}CHRC(O)$-	Glutathione	8.4	Very slow	Tamba and Quintiliani (1984)
$^{\bullet}CH_2C(O)H$	Dithiothreitol	8.4	Very slow	Akhlaq et al. (1987b)
$^{\bullet}C(NH_2)[CH_3S(CH_3)_2]H$	Lipoic acid	8.8	$(1\text{-}2) \times 10^9$	Hiller and Asmus (1983)

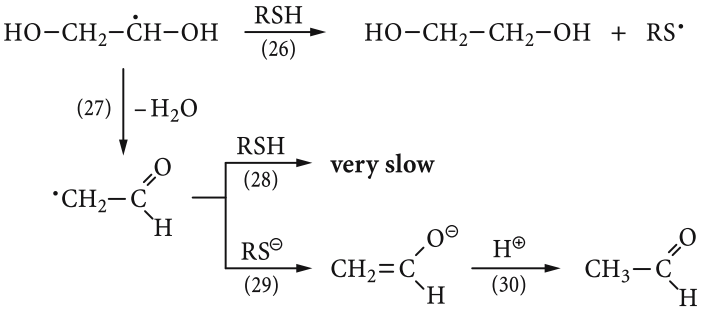
7.4.1 Hydrogen and Electron-Transfer Reactions by Thiols/Thiolate Ions

The S-H bond is weak (alkylmercaptans: BDE ≈ 366 kJ mol^{-1}, thiophenol: BDE ≈ 330 kJ mol^{-1}; Armstrong 1999), and for this reason thiols can serve as H-donors [reaction (26)]. Thus thiols can play an important role in the repair of free-radical-induced damage (for some early studies see Adams et al. 1967, 1968, 1969). Some rate constants are compiled in Table 7.3. Compared to aqueous solutions, the rate of H-transfer by thiols is slower in organic solvents (Tronche et al. 1996).

It is seen from this table that the rate of reaction increases $^{\bullet}CH_2C(CH_3)_2OH < {}^{\bullet}CH_2OH < {}^{\bullet}C(CH_3)HOH < {}^{\bullet}C(CH_3)_2OH$ despite the fact that the exothermicity of the reaction *decreases* in the same direction. This unexpected behavior has been discussed in terms of the charge and spin polarization in the transition state, as determined by the AIM analysis, and in terms of orbital interaction theory (Reid et al. 2002). Rate constants, calculated by transition state theory, were in good agreement with the experimental data.

Even in neutral solution, a small, but sometimes noticeable, fraction is present as thiolate ion (pK_a(thiophenol) = 6.6; Armstrong et al. 1996; pK_a(alkylmercaptans) ≈ 9.5; pK_a(GSH) = 9.2). The thiolates are reasonably good reducing agents and can act as electron donors.

It is seen from Table 7.3 that some 'oxidizing radicals' such as the formylmethyl radical are not reduced effectively by thiols. They are, however, readily reduced by thiolate ions. A good example for the different reactivities of thiols/thiolate ions toward reducing and oxidizing radicals is the ethylene glycol system (Akhlaq et al. 1987b). The radical derived by H-abstraction is a 'reducing' α-hydroxyalkyl radical and hence rapidly reduced by a thiol. This radical can eliminate spontaneously and acid/base-catalyzed water yielding the 'oxidizing' formylmethyl radical [reaction (27); Chap. 6.9]. The latter no longer reacts with the thiol at an appreciable rate [reaction (28); $k << 10^7$ dm^3 mol^{-1} s^{-1}] but rapidly with the thiolate ion [reaction (29); $k = 3.5 \times 10^8$ dm^3 mol^{-1} s^{-1}; with DTT at pH 11.1], possibly by ET. Although acetaldehyde is the final product [overall reaction (30)], its enol will be an intermediate for kinetic reasons (faster protonation at oxygen than at carbon; see Chap. 6.2).



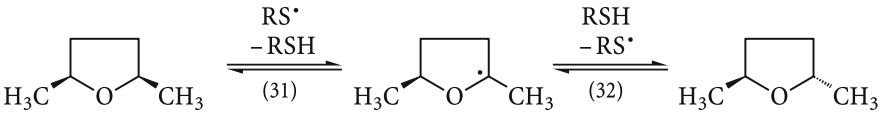
The repair of the •OH-induced radicals derived from carbohydrates is rarely 100% on the pulse radiolysis time scale (Held et al. 1985). This is in agreement with the above. It is especially low for ribose-5-phosphate (60%), where the very rapid phosphate elimination increases the yield of 'oxidizing' radicals.

The (oxidizing) peroxyl radicals behave in a similar fashion. They do not react readily with thiols, not even with thiophenols whose H atoms are very weakly bound, but they are readily reduced by the corresponding thiolate ion in contrast to alkyl radicals which are poor electron acceptors and hence do not react with thiolate ions (Simic and Hunter 1986).

7.4.2 H-Abstraction by the Thiyl Radical

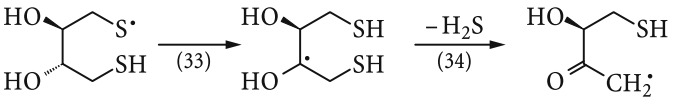
Reaction (26) is, in principle, reversible, and if the C-H BDE of the product RH is close to that of the S-H BDE of the thiol the reverse reaction becomes of importance. This has first been shown for 2,5-dimethyltetrahydrofurane, where thiyl radicals induce a *cis-trans* isomerization via a chain reaction [reactions

(31) and (32); Akhlaq et al. 1987a; for similar observations with tetrahydrofufuryl acetate, see Cai and Roberts 1998].

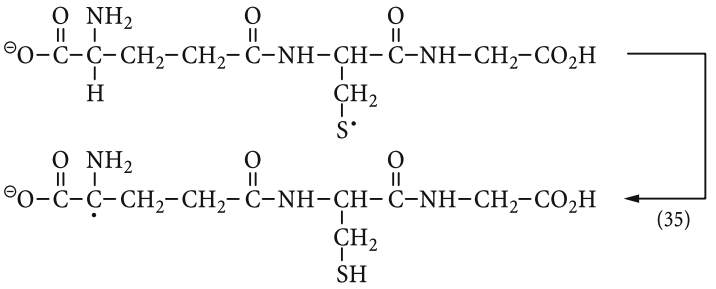


From pulse radiolysis experiments the rate of H-donation by the thiol has been determined at 7×10^8 dm^3 mol^{-1} s^{-1}, while from the *cis-trans* isomerization data the rate of the H-abstraction by the thiyl radical has been calculated at ca. 10^4 dm^3 mol^{-1} s^{-1} (Akhlaq et al. 1987a). Similar data have been reported for the reaction of thiyl radicals with alcohols [k(•C(CH$_3$)$_2$OH + PenSH) = 1.2×10^8 dm^3 mol^{-1} s^{-1}k; (PenS• + 2-PrOH) = 1.4×10^4 dm^3 mol^{-1} s^{-1}; Schöneich et al. 1989, 1990] and with carbohydrates (2-deoxyribose: 2.7×10^4 dm^3 mol^{-1} s^{-1}; Pogocki and Schöneich 2001). Thus, in these systems, the rate of H-donation by the thiol is four to five orders of magnitude faster than the rate of H-abstraction by the thiol, that is, these data are not in contradiction with the general conclusion that thiols do reduce *C*-centered free-radicals and that the reverse reaction is usually not observed. On the other hand, when the C-H BDE is lower such as in the case of the pentadienylic position in polyunsaturated fatty acids, the rate of H-abstraction by thiyl becomes very fast [k(RS• + RH) ≈ 10^7 dm^3 mol^{-1} s^{-1}; Schöneich and Asmus 1990; Schöneich et al. 1992], and it is not very likely that the reverse ('repair') reaction is of any importance here.

An H-transfer may also occurs intramolecularly such as in DDT via a five-membered transition state [reaction (33);Akhlaq and von Sonntag 1986]. In the given case, an H_2S forming chain reaction is induced [cf. reaction (34) followed by the (slow) H-abstraction of the thus-formed radical from DTT] which comes to a halt when the thiyl radical is complexed as the disulfide radical anion at higher pH values [cf. reaction (40)].



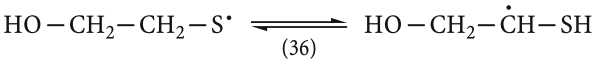
With GSH carbon-centered besides thiyl radical are formed upon •OH attack, notably at pH > 7 (Sjöberg et al. 1982; Eriksen and Fransson 1988). It has been shown subsequently that this is due to an intramolecular H-transfer [reaction (35); Grierson et al. 1992]. When the a-NH$_2$ group is no longer protonated as in neutral solution the C-H is only weakly bound and equilibrium (35) is shifted to the side of the *C*-centered radical.



When the amino group is fully deprotonated, the rate of the H-transfer is ~1.5 × 10^5 s^{-1}, but also around pH 7 the reaction is still fast, at the ms timescale (for a quantum mechanical study see Rauk et al. 2001). Upon the decay of the amnioalkyl radicals formed in reaction (35) ammonia as formed in a yield that points to disproportionation as the major process (Zhao et al. 1997). The fact that the aminoalkyl radical is the thermodynamically favored species does not mean that the repair of DNA radicals by GSH (Chap. 12.11) is not due to its action as a thiol. As with many other examples described in this book, the much faster kinetics that lead to a metastable intermediate (here: the formation of the thiyl radical) rather than the thermodynamics as determined by the most stable species (here: the aminoalkyl radical) determine the pathway the the reaction. In fact, the C-H BDE of the peptide linkage is lower than the S-H BDE and repair of DNA radicals by peptides, e.g., proteins would be thermodynamically favored over a repair by thiols but this reaction is retarded kinetically (Reid et al. 2003a,b).

Considering the ability of thiyl radicals to abstract α-alkoxy-C-H, the question comes to mind, whether they might induce SSBs in DNA by abstracting the *C*(4′)-H or induce β→α-anomerizations at *C*(1′) after *C*(1′)-H-abstraction. To our knowledge, this has never been shown, but conditions may be thought of, where this reaction can proceed in vitro. In vivo, GSH is the dominating thiol. Its thiyl radical has only a rather short intrinsic lifetime [reaction (35)], and this will strongly reduce the probability of attacking the sugar moiety. Moreover, GSH is negatively charged and so is GS•. This additionally reduces the rate of reaction notably within the minor groove with its high negative charge density.

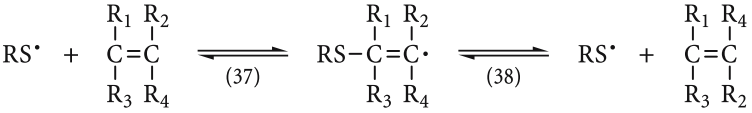
In contrast to their oxygen- and nitrogen-centered analogs [reactions (6) and (25)], 1,2-H-shift reactions of thiyl radicals are not only slow but the equilibrium lies practically fully on the side of the thiyl radical [reaction (36); Zhang et al. 1994; Naumov and von Sonntag 2005].



This equilibrium is only of any consequence when one of these two radicals does not react (or reacts reversibly) with a given substrate, while the other does (O_2 is an example; Chap. 8.2).

7.4.3
Addition of Thiyl Radicals to C-C Double Bonds

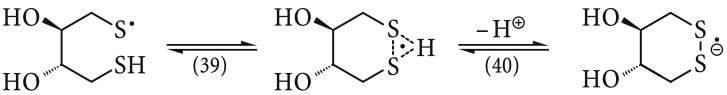
Thiyl radicals add to C-C double bonds, but this reaction is strongly reversible (Ito and Matsuda 1979, 1981, 1982; Ito 1992, 1995). As a consequence, *cis-trans* isomerization may occur [reactions (37) and (38)].



Typical examples are unsaturated fatty acids (Ferreri et al. 1999; Sprinz et al. 2000, 2001; Adhikari et al. 2001). The equilibrium constants for the oleic and linoleic systems are in the order of 10 dm^3 mol^{-1} and the reverse reaction in the order of 10^6 s^{-1} (Sprinz et al. 2000 and Sprinz, pers. comm.). In polyunsaturated fatty acids, such isomerizations could, in principle, also occur by an H-abstraction/H-donation mechanism as discussed above. However, the rate of H-donation of RSH to the pentadienylic radicals must be very low (see above), and isomerization has been considered to occur only by the addition/elimination pathway (Sprinz et al. 2000). With the nucleobases, any thiyl addition can only be detected when the short-lived adduct is trapped by a fast reaction (Chap. 10.10).

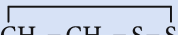
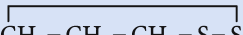
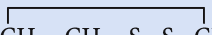
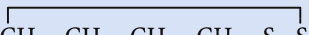
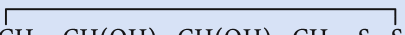
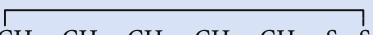
7.4.4
Three-Electron-Bonded Intermediates in Sulfur Radical Reactions

Sulfur free-radical chemistry is largely governed by the ability of sulfur to form three-electron bonded intermediates. A case in point is the complexation of a thiyl radical with a thiolate ion (for an analogy with the halide and other pseudohalide systems, see Chap. 5.2). These disulfide radical anions are characterized by strong absorptions in the UV-Vis (Adams et al. 1967). Complexation can occur both intermolecularly as well as intramolecularly. For GSH, for example, the stability constant of the disulfide radical anion is 2900 dm^3 mol^{-1} (Mezyk 1996a). The protonated disulfide radical anion is not stable, but such intermediates are known in the cases of the intramolecular complexes [reactions (39) and (40); Akhlaq and von Sonntag 1987].



The protonated intramolecular complexes absorb at slightly shorter wavelengths, while the thiyl radicals do not absorb in the wavelength region of interest. The five-membered complex is the most stable one (von Sonntag 1990), and its stability decreases both to smaller and larger ring sizes (Table 7.4). It can be seen from this table that in the case of the 4-membered ring system the absorption maximum is shifted significantly to longer wavelength than in the other

Table 7.4. Absorption maxima and molar absorption coefficients of cyclic disulfide radical anions and their protonated forms. According to von Sonntag (1990). The lipoic acid value has been taken from Hoffman and Hayon (1972). Note that the equilibria (cf. apparent pK_a values) include the ring-open forms which are not detectable

					pK_a
	λ_{max}	ε	λ_{max}	ε	
	500	2900	460	240	6.7
	400	6800	380	4910	6.6
	410	9200	385	6900	5.85
	400	4540	380	290	7.0
	390	6600	380	450	5.2
	425	3830	370	155	9.4

systems. This is in keeping with a weaker three-electron bond due to ring strain (Asmus 1979).

The equilibria of a series of sulfhydryl groups containing amino acids have been investigated (Mezyk 1995, 1996a,b), and details governing these equilibria are now reasonably well understood (Mezyk and Armstrong 1999).

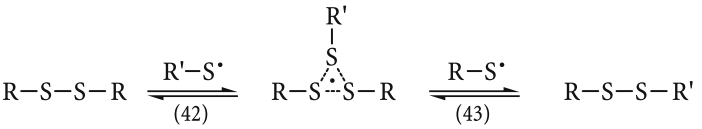
Metallothioneins are small ubiquitous oligopeptides containing a high proportion of cysteine residues but no disulfide bonds (Tsunoo et al. 1978; Suzuki and Maitani 1983). Mammalian metallothionins are made up of 61 amino acids, 20 of which are cysteine (Hamer 1986; for their protective effects see Chap. 12.10). Despite this, disulfide radical anion formation is mostly bimolecular (Fang et al. 1995).

The disulfide radical anions are also formed upon the reduction of the disulfide by $CO_2^{\bullet-}$ [reaction (41)] and the thymine radical anion (Willson 1970; Elliot et al. 1984). For example, the reducing hydroxymethyl radical can reduce the disulfide (Anderson et al. 1986), but the rate is only fast when the hydroxymethyl radical is deprotonated (Akhlaq et al. 1989) and thus its redox potential decreased.

$$RSSR + {}^{\bullet}CH_2O^- \rightarrow [RSSR]^{\bullet-} + CH_2O \qquad (41)$$

Disulfide radical anions are rather strong reductants (E = –1.7 V; Wardman 1989), and it has hence been proposed that in cellular systems these intermediates may contribute to the repair of DNA radicals (Prütz 1989).

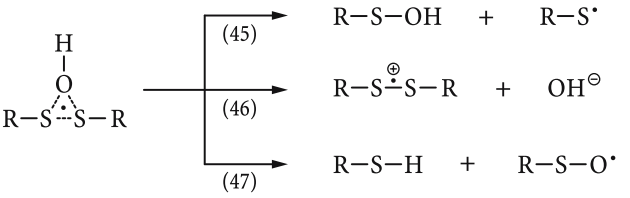
Similar three-electron-bonded intermediates must also be formed in the well-known thiyl radical-induced scrambling of disulfides (Owen and Ellis 1973). Mechanistic details have been studied using the pulse radiolysis technique which allowed the identification the three-bonded intermediate and the determination of some equilibrium constants, but also gave evidence that this system may be more complex than described by reactions (42) and (43) (Bonifacic and Asmus 1984).



The rate constant of the similar reaction (44) varies strongly with the substituent R and decreases in the case of R′ = phenyl by two orders of magnitude on going from R = methyl to R = *tert*-butyl, R = 2-propyl lying in between (Pryor and Smith 1970).

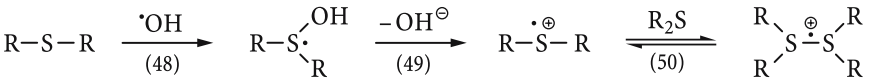
$$RSSR + {}^{\bullet}R' \rightarrow RSSR' + R^{\bullet} \qquad (44)$$

Addition to the disulfide function is also observed with •OH (Bonifacic et al. 1975b). But here, in addition to the substitution reaction (45), generation of a disulfide radical cation and a hydroxide ion is also observed [reaction (46)]. The high solvation energy of the latter certainly provides additional driving force. There is EPR evidence that RSO• is also formed [reaction (47); Gilbert et al. 1975]. In dithiodipropionic acid and in cystine, the thiol is indeed a major product (30-40%; Elliot et al. 1981).



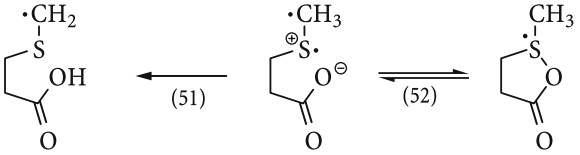
Cationic species are also formed when sulfides (Meissner et al. 1967; Adams 1970; Bonifacic et al. 1975a; Janata et al. 1980; Hiller et al. 1981; Davies et al. 1984; Ramakrishna Rao et al. 1984) or thioureas (Wang et al. 1999; Schuchmann et al. 2000) react with •OH. Especially stable are the dimeric radical cations [reactions (48)–(50)]. In the case of thiourea, the high stability of the dimeric radical cation may contribute to the driving force which leads, in acid solution, to its forma-

tion even by reducing radicals such as the H atom and α-hydroxyalkyl radicals (Schuchmann et al. 2000).



In addition to the complexation by the parent, the sulfide radical cation can undergo a deprotonation at a neighboring carbon [cf. reaction (51)]. Also, in the case of thiourea, deprotonation at nitrogen in basic solution competes with a complexation by the parent (Wang et al. 1999). This reaction is, of course, not given by its *N*,*N*,*N*′,*N*′-tetramethyl derivative.

The sulfide radical cation can also complex with neighboring hydroxy, alkoxy and carboxylate groups (e.g., Glass et al. 1984; Asmus et al. 1985; Chatgilialoglu et al. 1985; Mahling et al. 1987; Pogocki and Schöneich 2002). The reaction with the latter that gives rise to a sulfuranyl radical is shown in reaction (52).



All these radical cations are fairly strong oxidants. For example, the monomeric methionine radical cation is likely to propagate oxidative damage in peptides (Rauk et al. 2000). Yet in cellular systems, thiourea has radiation-protective properties (Bacq 1965). This may be accounted for considering that the oxidative power of its dimeric radical is not high enough to oxidize even G; it reacts only reasonably fast when G is deprotonated (Schuchmann et al. 2000), and this is a non-relevant situation in DNA.

Obviously, these sulfur-centered radical cations are good sinks for $O_2^{\bullet-}$, and, for example, disulfide radical cations give rise to sulfoxides in a very fast reaction [reaction (53), $k = 1.6 \times 10^{10}$ dm^3 mol^{-1} s^{-1}; Bonifacic et al. 2000b].

$$R_2SSR_2^{\bullet+} + O_2^{\bullet-} \rightarrow 2\,R_2S{=}O \qquad (53)$$

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8.1 General Remarks

Dioxygen, O_2, is omnipresent in our environment. Its solubility in water is 1.35×10^{-3} mol dm^{-3} at 20 °C (air-saturated: 2.8×10^{-4} mol dm^{-3}), and at 37 °C it drops to 77% of these values. Most experiments that are reported in the literature to have been carried out under ambient conditions will contain that much O_2. However, as a free-radical-induced reaction continues to proceed it is likely to consume O_2, and due to the low prevailing O_2 concentration it may become consumed before the reaction has been stopped. This can lead to considerable artifacts.

It is often assumed that the O_2 concentration in a cellular environment is the same as that in dilute aqueous solution. This assumption does not seem to be correct, not only because O_2 may be consumed by metabolic processes, and its rediffusion to the target site, e.g. to the nucleus, may be too slow to replenish the O_2 concentration to saturation level. More importantly, the solubility of O_2 in such highly concentrated solutions of proteins and nucleic acids that prevail in the nucleus is extremely low, that is, only a small fraction of that in water (Zander 1976a,b).

In radiobiology, O_2 has a sensitizing effect on cell survival and other parameters (Chap. 12.11). Moreover, the superoxide radical, $O_2^{\bullet-}$, may trigger important cellular reactions (although many of its reactions are slow; for a review see Bielski and Gebicki 1970). For example, there is increasing evidence that $O_2^{\bullet-}$ may play a role in the intercellular induction of apoptosis (Bauer 2000; Engelmann et al. 2000; Herdener et al. 2000).

8.2 Kinetics of the Reaction of Free Radicals with Oxygen

Details of the formation and decay of peroxyl radicals in an aqueous environment have been reviewed in some detail (von Sonntag and Schuchmann 1991, 1997; Schuchmann and von Sonntag 1997), and its suffices here to report only the most important points.

Most organic radicals react practically irreversibly with O_2 at close to diffusion-controlled rates (typically at 2×10^9 dm^3 mol^{-1} s^{-1}), and in air-saturated aqueous solutions the lifetime of these radicals will only be ~2 μs [reaction (1); for a compilation of rate constants see Neta et al. 1990].

$$R^{\bullet} + O_2 \rightleftharpoons RO_2^{\bullet} \qquad (1)$$

Not all encounters in the reaction of O_2 with e_{aq}^- lead to $O_2^{\bullet-}$, and the appropriate spin factor for this is 2/3 because of the large zero-field splitting of triplet O_2 (Schmidt et al. 1995). Similarly, spin dephasing is observed for the reaction of $H^{\bullet}$ with O_2 (Han and Bartels 1994), and this may apply also to other $R^{\bullet}$ plus O_2 reactions.

The R-OO$^{\bullet}$ BDEs for alkylperoxyl radicals are around 125 kJ mol^{-1}, for vinyl- and aryl-type peroxyl radicals they are even higher by about 63 kJ mol^{-1} (Kranen-

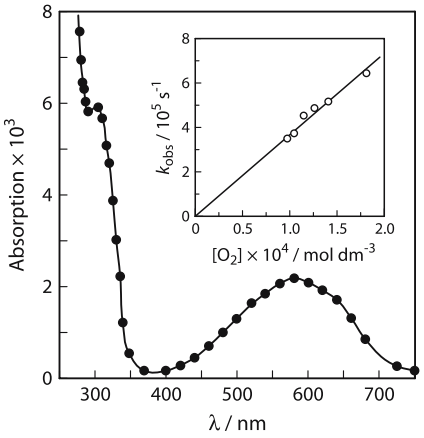


Fig. 8.1. Pulse radiolysis of Ar/O_2 (9:1)-saturated aqueous solution of tetrachloroethene. Absorption spectrum of the trichlorovinylperoxyl radical taken after completion of the reaction. *Inset* Rate of buildup of the trichlorovinylperoxyl radical as a function of the O_2 concentration. (Source: Mertens and von Sonntag 1994, with permission)

burg et al. 2000). Only for the cyclohexadienyl radical, it is as low as 25 kJ mol^{-1}, and this leads to noticeable reversibility (for DFT calculations see Naumov and von Sonntag 2005). For the related pentadienylperoxyl radical a BDE of 56 kJ mol^{-1} has been determined for the gas phase (Zils 2000; Zils et al. 2001).

As a rule of thumb, peroxyl radicals absorb at shorter wavelength and have lower and less structured absorptions than their parent radicals. There are only a few exceptions: vinyl- and phenyl-type peroxyl radicals (Alfassi et al. 1994, 1995; Mertens and von Sonntag 1994; Fang et al. 1995b; Khaikin and Neta 1995) as well as thiylperoxyl radicals (Jayson et al. 1971; Tamba et al. 1986; Zhang et al. 1994) absorb in the near UV and visible, cf. Fig. 8.1, while their parent radicals have barely any absorption in the wavelength region of interest (for a quantum-chemical study of peroxyl radical absorption spectra see Naumov and von Sonntag 2005).

In the present context of nucleic acid free-radical chemistry, such a strong absorption in the visible of a vinylic peroxyl radical is observed in the case of the uracil-5-peroxyl radical (λ_{max} = 570 nm; Mertens and von Sonntag 1994) which plays a role in the free-radical chemistry of 5BrUra.

When the C-OO$^\bullet$ bond is weak, the R$^\bullet$ + O_2/ROO$^\bullet$ system becomes reversible (k_{-1} now being quite fast). To date, examples of reversibility at room temperature has only been found for pentadienyl-type radicals (Chan et al. 1978; Pan and von Sonntag 1990; Pan et al. 1993; Fang et al. 1995a). Such radicals are formed, when a pentadienylic hydrogen is abstracted from polyunsaturated fatty acids or when $^\bullet$OH adds to aromatic compounds (forming hydroxycyclohexadienyl radicals). Hydroxycyclohexadienyl radicals absorb strongly near 310 nm (325 nm in the case of the one derived from anisole, Fig. 8.2), while the corresponding peroxyl radicals absorb only weakly at this wavelength. Due to the reversibility of the re-

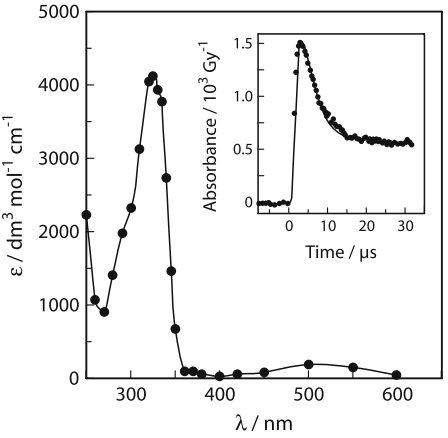


Fig. 8.2. Pulse radiolysis of N_2O-saturated aqueous solutions of anisole. Spectrum of the anisole-derived hydroxycyclohexadienyl radicals. *Inset* shows their decay in the presence of 15% O_2 and formation of the "plateau". (Source: Fang et al. 1995a, with permission)

action, the absorbance of the hydroxycyclohexadienyl radicals does not decay to the low level that would be given by the absorption of the remaining hydroxycyclohexadienylperoxyl radicals alone, but settles a higher "plateau" value (Fig. 8.2, inset). The height of this "plateau" decreases with increasing O_2 concentration.

Subsequent to the rapid attainment of this near-equilibrium situation, the radicals decay more slowly by both unimolecular and bimolecular processes (discussed below). For this simple case, it can be shown that when the decay process is much slower than the rates of the forward and reverse reactions, the observed rate constant for the disappearance of $R^\bullet$ is given by the expression $k_{obs} = k_{forward}[O_2] + k_{reverse}$. When k_{obs} of the decay of the hydroxycyclohexadienyl radicals is plotted as a function of the O_2 concentration, the slope represents the rate constant of the forward reaction, and the intercept that of the reverse reaction (Fig. 8.3).

Stability constants of a number of differently substituted hydroxycyclohexadienyl peroxyl radicals have been obtained from such data (for a compilation see von Sonntag and Schuchmann 1997).

The thiyl/thiylperoxyl radical system is also reversible [reaction (2); Tamba et al. 1986; Zhang et al. 1994], although the thiylperoxyl radicals are stabilized by an intramolecular charge transfer (Razskazovskii et al. 1995). The evaluation of the equilibrium constant is not straightforward because the thiylperoxyl radical undergoes a thermal but also a light-induced (Sevilla et al. 1990a) rearrangement into the much more stable sulfonyl radical [reaction (3)] which is subsequently converted into the corresponding peroxyl radical [reaction (4)]. In addition, equilibrium (5) has to be taken into account, although this equilibrium lies largely on the side of the thiyl radical (Zhang et al. 1994; for DFT calculations see Naumov and von Sonntag 2005).

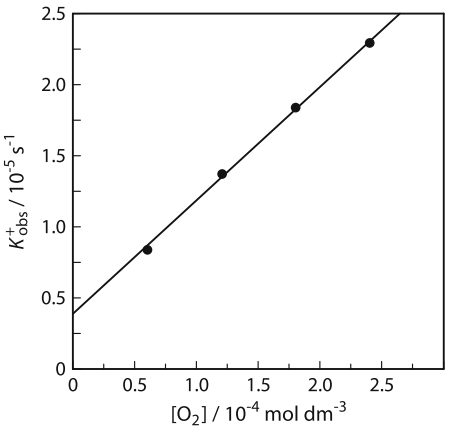
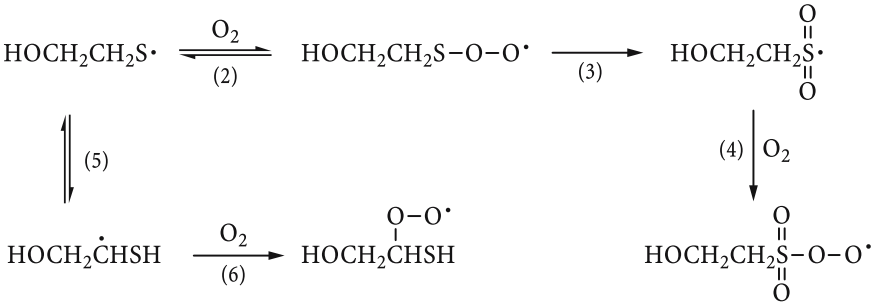


Fig. 8.3. Pulse radiolysis of N_2O-saturated aqueous solutions of anisole. Plot of the rate of the decay of the anisole-derived hydroxycyclohexadienyl radicals as a function of the O_2 concentration. (Source: Fang et al. 1995a, with permission)

While most carbon-centered radicals react fast or at least reversibly with O_2, some highly conjugated radicals that have considerable spin density at a heteroatom do not react with O_2 (for the reactions of heteroatom-centered radicals with



O_2, see Schuchmann and von Sonntag 1997). Typical examples are the phenoxyl radicals (Hunter et al. 1989; Jin et al. 1993) and the vinylogs of phenoxyl radicals (Benjan et al. 2001). They only show some reactivity when the electron density in the ring is increased by electron donating substituents (Wang et al. 1994). Other examples are the tryptophan-derived radical (Fang et al. 1998) and, most relevant to DNA, the guanyl radical, G$^{\bullet}$ (von Sonntag 1994).

8.3 Geometries, Spin Densities, Oxidative Power and pK_a Values of Peroxyl Radicals

Equilibrium geometries, harmonic vibrational frequencies, dipole moments and hyperfine couplings for a series of peroxyl radicals have been calculated by an ab initio method (Besler et al. 1986). The spin density of ^{17}O-enriched peroxyl radicals correlates well with the Taft substituent parameter σ* and the ET rate with strong reductants (Sevilla et al. 1990b; for a theoretical study see Raiti and Sevilla 1999; for further studies on the reduction of peroxyl radicals see Packer et al. 1980; Alfassi et al. 1987; Asmus et al. 1988; Schuchmann and von Sonntag 1988; Neta et al. 1989; El-Agamey and McGarvey 2002). The redox potential of simple alkylperoxyl radicals is $E^7 = 0.77$ V; it is substantially increased by electron-withdrawing substituents [$E^7(CCl_3OO^{\bullet}) = 1.15$ V, $E^7(RC(O)OO^{\bullet}) = 1.6$ V; Merényi et al. 1994; for arylperoxyl radicals, see Alfassi et al. 1995]. The highly chlorinated peroxyl radicals oxidize some nucleobase anions (at high pH) quite effectively (Kapoor and Gopinathan 1992), but their reduction potential is too low to oxidize even Gua at substantial rates in neutral solutions.

A similar gradation is observed, when $O_2^{\bullet-}$ is the reductant. The rate of the reaction of the most powerful peroxyl radical, the acetylperoxyl radical, with $O_2^{\bullet-}$ is close to diffusion-controlled [reaction (7); $k \approx 10^9$ dm^3 mol^{-1} s^{-1}; Schuchmann and von Sonntag 1988], while the α-hydroxyethylperoxyl radical reacts with $O_2^{\bullet-}$ merely with a rate constant near 10^7 dm^3 mol^{-1} s^{-1} (Bothe et al. 1983).

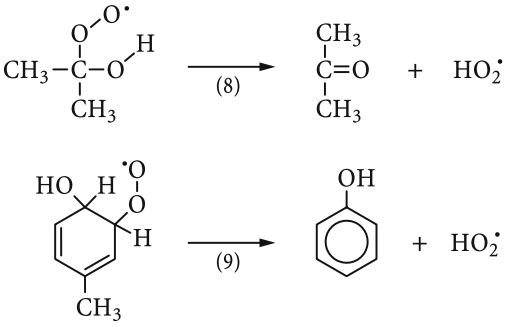
$$CH_3C(O)OO^{\bullet} + O_2^{\bullet-} \rightarrow CH_3C(O)OO^{-} + O_2 \qquad (7)$$

The peroxyl radical group is among the most strongly electron-withdrawing substituents (Schuchmann et al. 1989), and in a plot of the pK_a values of substituted acetic acids vs. the Taft σ* constant its value of 3.7 falls in between those of the cyano and nitro groups. This strong electron withdrawing property of the peroxyl radical function strongly lowers, of course, the pK_a value of the peroxyl radical compared to that of the parent compound (acetic acid: Schuchmann et al. 1989; malonic acid: Schuchmann et al. 2000; formamide: Muñoz et al. 2000). pK_a values of peroxyl radicals can be predicted (Muñoz et al. 2000) using the above Taft σ* value and the compiled Taft parameters (Perrin et al. 1981).

8.4 $HO_2^{\bullet}/O_2^{\bullet-}$-Elimination Reactions

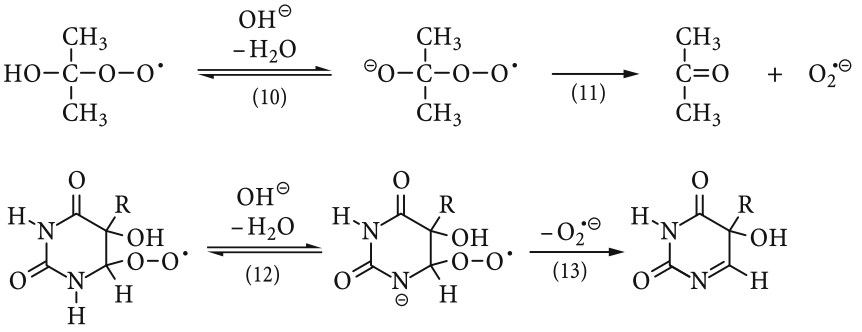
Peroxyl radicals undergo a number of unimolecular processes. The most ubiquitous one is the elimination of $HO_2^{\bullet}/O_2^{\bullet-}$. They govern the peroxyl free-radical chemistry of carbohydrates (von Sonntag 1980) and prevent their autoxidation in aqueous solution (Schuchmann and von Sonntag 1978). The driving force of the $HO_2^{\bullet}$ elimination is due to the formation of a double bond [e.g., reactions (8) and (9); $k_8 = 650$ s^{-1}; $k_9 = 800$ s^{-1}; Bothe et al. 1977, 1983; Pan and von Sonntag 1990; Wang et al. 1993; Pan et al. 1993; Fang et al. 1995a]. It has been suggested

that this reaction occurs via a five-membered transition state (Bothe et al. 1977), and largely due to steric reasons only the 1,2- but not the 1,4-hydroxycyclohexadienylperoxyl radical eliminates $HO_2^\bullet$ (Pan et al. 1993).



The rate of $HO_2^\bullet$ elimination from α-hydroxyalkylperoxyl radicals strongly depends on the flanking substituents that also govern the strength of the resulting C-O double bond (for a compilation see von Sonntag and Schuchmann 1997).

The $O_2^{\bullet-}$-elimination reactions may be divided into three groups. Those peroxyl radicals that have an –OH or –NH function in the α-position make up the first group. Such peroxyl radicals play a major role in nucleobase peroxyl radical chemistry [cf. reactions (12) and (13)]. Upon deprotonation at the heteroatom by OH^- [reactions (10) and (12)], the peroxyl radical anion is formed (cf. the enhancement of the acidity of the functions α to the peroxyl group discussed above; for the thermodynamics of the various equilibria that are involved in these reactions see Goldstein et al. 2002). As before, the driving force for the elimination reaction is the formation of a double bond [in addition to the energy gain by the formation of the stabilized $O_2^{\bullet-}$ radical [cf. reactions (11) and (13)].



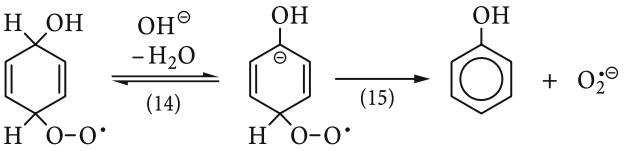
The peroxyl radical anion formed in reaction (10) has an immeasurably short ($<< 10^{-6}$ s^{-1}) lifetime, i.e., k_{11} is much larger than $k_{-10} \times [H_2O]$, and even at high $[OH^-]$ the rate of acetone formation is essentially given by $k_{10} \times [OH^-]$ (Bothe et al. 1977). The situation is similar for other α-hydroxyalkylperoxyl radical anions (Rabani et al. 1974; Ilan et al. 1976; Bothe et al. 1983) with the exception

Table 8.1. pK_a values of radical derived from glycine and alanine anhydrides. Rates of $O_2^{\bullet-}$-elimination of their peroxyl radical anions. (Mieden and von Sonntag 1989; Mieden et al. 1993)

Parameter	Glycine anhydride	Alanine anhydride
pK_a of parent radical	9.8	10.6
pK_a of peroxyl radical	10.7	11.2
$k(O_2^{\bullet-}$ elimination) s^{-1}	1.6×10^5	3.9×10^6

of the peroxyl radical derived from hydroxymalonic acid, where the $O_2^{\bullet-}$-elimination from the peroxyl radical anion is not much faster than the $HO_2^{\bullet}$ elimination of from the protonated form (Schuchmann et al. 1995). Also relatively long lifetimes with respect to $O_2^{\bullet-}$-elimination are given by the peroxyl radical anions derived from uracil (Schuchmann and von Sonntag 1983; Schuchmann et al. 1984), atrazine (Tauber and von Sonntag 2000), formamide (Muñoz et al. 2000) and cyclic dipeptides (Mieden et al. 1993). For the latter system more detailed data are available (Table 8.1). Substitution of the prototype radical by an electron donating methyl group raises the pK_a values. For the same reason, the rate of $O_2^{\bullet-}$-release is enhanced by methyl substitution.

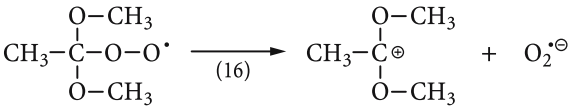
The second group is related to the first group, but here a distant *carbon*-bound hydrogen must be removed. A case in point is the 1-hydroxycyclohexadienyl-4-peroxyl radical [reactions (14) and (15)].



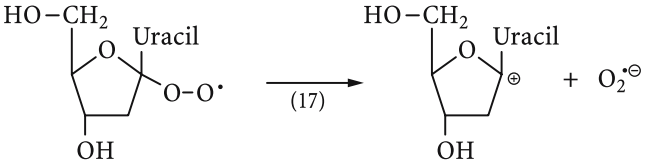
Whereas base-induced deprotonation at a heteroatom is very fast (practically diffusion-controlled), deprotonation at carbon is generally much slower (Eigen et al. 1964, 1965). Thus, this type of $O_2^{\bullet-}$-elimination is observed at higher pH values compared to the reactions discussed before. The elimination of $HO_2^{\bullet}$ is subject to steric restrictions, but the OH^--induced $O_2^{\bullet-}$-elimination is not, and at high pH all hydroxycyclohexadienylperoxyl radicals eliminate $O_2^{\bullet-}$ bringing the phenolate yield close to 100% [reactions (9) and (14)/(15)]; competing reactions (see below) are thereby suppressed.

Experiments are often and carried out in the presence of buffer. Thus, it is important to note that the $O_2^{\bullet-}$-elimination of α-hydroxyalkylperoxyl radicals is not only induced by OH^- but also by buffer, albeit with a much lower rate constant [1-hydroxyethylperoxyl radical: $k(OH^-) = 4 \times 10^9$ dm^3 mol^{-1} s^{-1}; $k(HPO_4^{2-}) = 4 \times 10^6$ dm^3 mol^1 s^{-1}; Bothe et al. 1983].

The third class of peroxyl radicals is characterized by a dissociation into a carbocation and $O_2^{\bullet-}$. One example has been unequivocally established to date [reaction (16); $k = 6.5 \times 10^4$ s^{-1}; Schuchmann et al. 1990].



Here, the driving force is provided by the stabilization of the cation by the adjacent electron-donating groups. It is typical for ionic dissociation processes that the rates depend strongly on the stabilization energies of the ions formed, and it is hence not surprising that the peroxyl radicals derived from diisopropylether (Schuchmann and von Sonntag 1987) and 1,3-dioxane (Nese et al. 1995) do not display a similarly fast $O_2^{\bullet-}$-elimination, as the former lacks the second alkoxyl function and the latter the methyl substituent. It has been suggested that also in the case of the *C*(1′) nucleoside radicals (formed through the photolysis of the pivaloyl derivative) this reaction plays a role [reaction (17); Emanuel et al. 1999]. However, the observation that the radicals generated in the photolysis of di-*tert*-butylketone undergo the same reactions has to be taken as a caveat.



In reactions (18) and (19) (Das et al. 1987), no peroxyl radical intermediates have been observed, but if they existed, they would compound among this group.

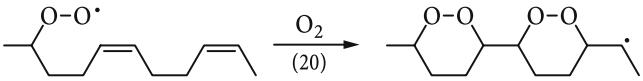
$$CO_2^{\bullet-} + O_2 \rightarrow CO_2 + O_2^{\bullet-} \quad (18)$$

$$^{\bullet}CH_2N(CH_3)_2 + O_2 \rightarrow (CH_3)_2N{=}CH_2^+ + O_2^{\bullet-} \quad (19)$$

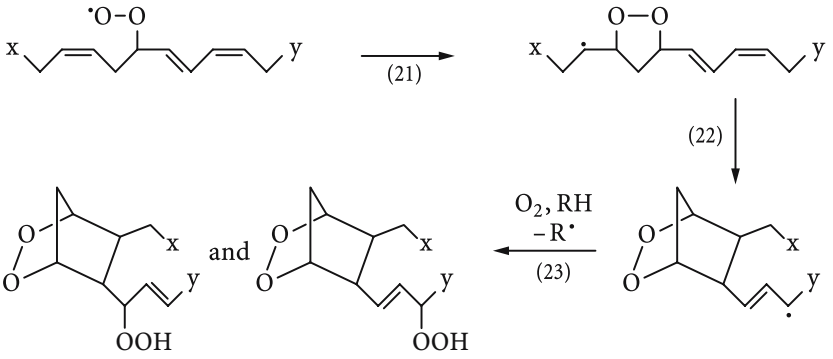
The rate constant of reaction (18) is close to diffusion controlled, and values ranging between 2×10^9 dm^3 mol^{-1} s^{-1} (Adams and Willson 1969; Buxton et al. 1976) and 4.2×10^9 dm^3 mol^{-1} s^{-1} (Ilan and Rabani 1976) have been reported.

8.5 Addition to the C–C Double Bond

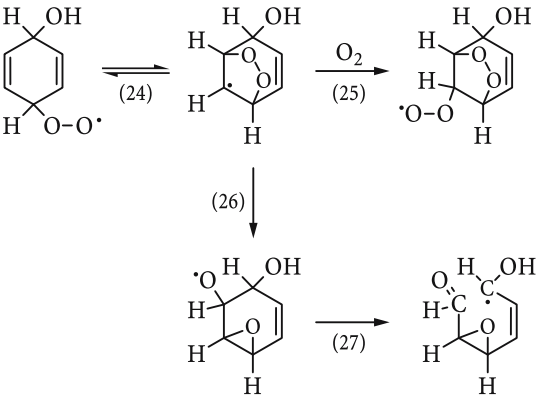
The addition of peroxyl radicals to double bonds is generally not very fast, and even with β-carotene the rate constant is less than 10^6 dm^3 mol^{-1} s^{-1} (Mortensen and Skibsted 1998). Nevertheless, peroxyl radicals of multi-unsaturated compounds have been reported to undergo chain-like peroxidation if the C-C double bonds are suitably disposed [cf. reaction (20); Porter et al. 1980, 1981].



The autoxidation of polyunsaturated fatty acids (cf. Porter et al. 1981) is usually monitored by the formation of "malonaldehyde" using the 2-thiobarbituric acid essay. This is carried out under rather severe conditions which decomposes its precursor. This malonaldehyde-like product is obviously formed via a cyclization reaction of a peroxyl radical, followed by other processes such as further cyclization and hydroperoxide formation [reactions (21)–(23)]. The resulting hydroperoxides may eliminate malonaldehyde upon a homolytic cleavage of the endoperoxidic intermediate (Pryor and Stanley 1975).

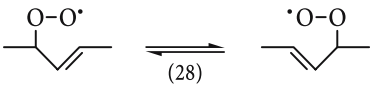


In the series of hydroxycyclohexadienylperoxyl radicals, one encounters the competition between the $HO_2^{\bullet}/O_2^{\bullet-}$ elimination leading to phenol [reactions (9) and (14)/(15)] and fragmentation of the ring (Pan et al. 1993). That latter has been attributed to an intramolecular addition of the peroxyl radical function to a diene double bond [reaction (24)]. This reaction is reversible [reaction (-24)], but when O_2 adds to the newly created carbon-centered radical the endoperoxidic function is locked in [reaction (25)]. In analogy to reaction (24), the first step of the trichloromethylperoxyl-radical-induced oxidation of indole is its addition to the indole *C*(2)-*C*(3) double bond (Shen et al. 1989).



In competition with O_2-addition [reaction (25)], the β-alkylperoxide species may undergo radical-induced cleavage of the peroxide function [reaction (26); Bloodworth et al. 1984; Phulkar et al. 1990].

In allylperoxyl radicals, allylic rearrangement leads to the 1,3-migration of the peroxyl function, with the corresponding shift of the double bond [reaction (28); Schenck et al. 1958].

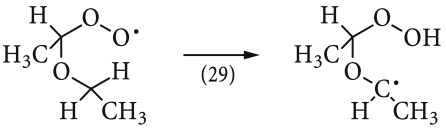


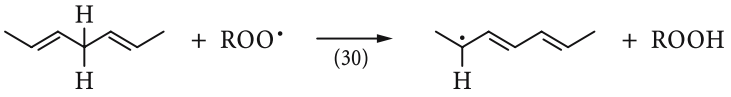
Evidence has been adduced that for many such systems, the apparently obvious cyclic intermediate 1,2-dioxanyl is not realized in the course of this rearrangement: an O_2–allylradical pair is postulated instead (Beckwith et al. 1989; Porter et al. 1994). Nevertheless, this cyclic structure has been invoked in the gas phase (Lodhi and Walker 1991; Bozzelli and Dean 1993), and an exothermicity of 96 kJ mol^{-1} has been computed for the formation of the cyclic intermediate relative to the level of allyl plus O_2 (Bozzelli and Dean 1993).

In DNA free-radical chemistry allylperoxyl radicals play a major role in the free-radical-induced oxidation of Thy. Thus far, this kind of rearrangement has not yet been observed (but also not especially looked for) in this system.

8.6 Intramolecular and Intermolecular H-Abstraction Reactions

The H-abstraction reactions of peroxyl radicals are related to ET discussed above, as in both cases the same final product is formed, a hydroperoxide. Mechanistically, these two processes are, of course, different. Hydrogen-abstraction reactions by peroxyl radical, including $HO_2^{\bullet}$, are common (cf. the autoxidation of polyunsaturated lipids;Hasegawa and Patterson 1978; Patterson and Hasegawa 1978; Patterson 1981; Porter et al. 1981; Gebicki and Bielski 1981; Barclay et al. 1989; Zhu and Sevilla 1990; Aikens and Dix 1991; Simic et al. 1992). This H-abstraction reaction may occur *intra*molecularly as well as *inter*molecularly [cf. reactions (29) and (30)].





The ROO-H BDE in hydroperoxides derived from weakly oxidizing peroxyl radicals is ca. 360-370 kJ mol^{-1} (Khursan and Martem'yanov 1991; Denisov and Denisova 1993), and, for a peroxyl radical reaction to occur at an appreciable rate, the C-H BDE of the donor must be sufficiently low. A case in point is aliphatic amines, but even with these the rate constants are rather low ($k < 500$ dm^3 mol^{-1} s^{-1} at 350 K; Dambrova et al. 2000). In DNA, the weakest C-H bond is that of the allylic hydrogen in Thy, and this is the preferred site of peroxyl radical attack (Razskazovskii and Sevilla 1996; Martini and Termini 1997). In poly(U), nucleobase peroxyl radicals abstract the *C*(2′)-H that is activated by the neighboring OH group (Chaps 9.4 and 11.2), but *C*(2′)-H abstraction in DNA is inefficient due to the higher BDE of this hydrogen.

Because of the often slow rate of reaction, even of intramolecular H-abstraction and a favorable six-membered transition state such as in reaction (29) ($k \approx 1$ s^{-1}; Schuchmann and von Sonntag 1982; for further examples see, e.g., Ulanski et al. 1996a,b), the reaction is only observed at a low steady-state of peroxyl radicals, that is, when the lifetime of peroxyl radical is long. Kinetic parameters with pre-exponential factors near 10^8 and activation energies varying from 30-60 kJ mol^{-1} have been reported for the reactions of peroxyl radicals with various alcohols (Denisov and Denisova 1993).

As a consequence of the reformation of the starting radical, a chain sets in [reactions (31)-(33)].

$$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet} \quad (31)$$

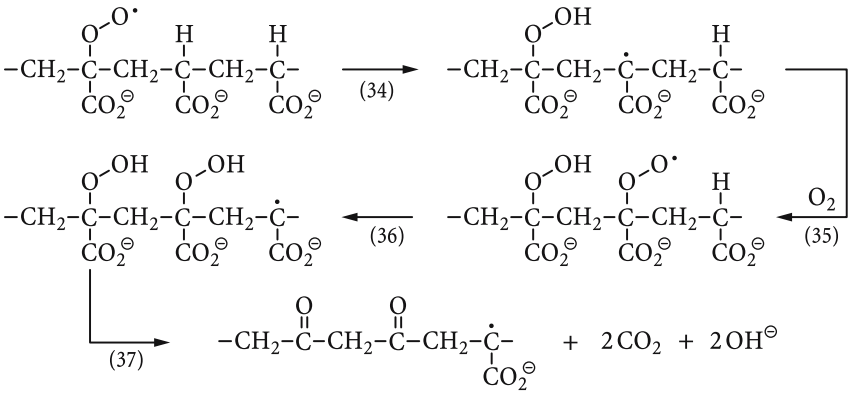
$$RO_2^{\bullet} + RH \rightarrow RO_2H + R^{\bullet} \quad (32)$$

$$2\,RO_2^{\bullet} \rightarrow \text{Products} \quad (33)$$

If this mechanism is strictly followed the chain length and hence the value of O_2-uptake (see below) increases linearly with the substrate concentration and (initiation rate)$^{-1/2}$ (i.e., in radiolytic studies the dose rate) and in charged polymers also on the pH (cf. Ulanski et al. 1996a). In polymers, the chain reaction may mainly proceed intramolecularly (Ulanski et al. 1996a; Janik et al. 2000). An example for an efficient intramolecular autoxidation is poly(acrylic acid) [reactions (34)−(36); Ulanski et al. 1996a]. In these autoxidation reactions, hydroperoxides are formed which, in some cases, are quite unstable [e.g. reaction (37); see also Leitzke et al. 2001].

In micellar systems, the chain peroxidation reaction only becomes effective at the critical micelle concentration where the substrate molecules aggregate thereby forming locally high concentrations (Gebicki and Allen 1969).

The hydroperoxides that are formed in all these autoxidation reactions all have low O-H BDEs. As a consequence, hydroperoxides are good H-donors in *non-aqueous* media, and they are often used to intercept radicals by H-donation. However, in water, where this function is hydrogen-bonded, the H-abstraction rates can drop by several orders of magnitude; the same phenomenon is observed with phenols (Das et al. 1981; Avila et al. 1995; Valgimigli et al. 1995; Banks et al. 1996; see also Ulanski et al. 1999).



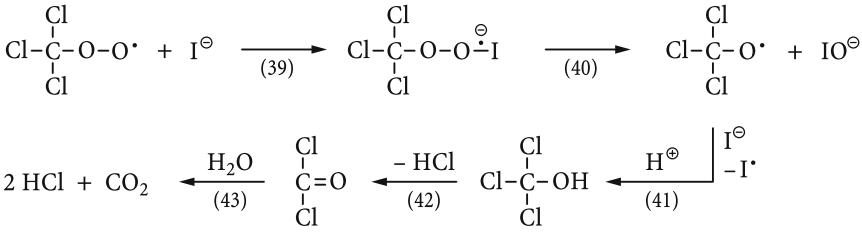
8.7 O-Transfer Reactions

O-transfer reactions of peroxyl radicals are sometimes referred to as two-electron reductions (Bonifacic et al. 1991; Schöneich et al. 1991; Merényi et al. 1994), in analogy to the one-electron reduction discussed above, although the reaction type is quite different. It requires the addition of the peroxyl radical to an electron-rich center and is thus reminiscent of the O-transfer in ozone reactions (Muñoz and von Sonntag 2000; Muñoz et al. 2001; Flyunt et al. 2003). In some cases, this complex may simply decay into an oxyl radical and an oxide as observed with diaryltellurides (Engman et al. 1995), phosphines (Engman et al. 1995) and disulfides [Schöneich et al. 1991; Bonifacic and Stefanic 2000; e.g., reaction (38)].

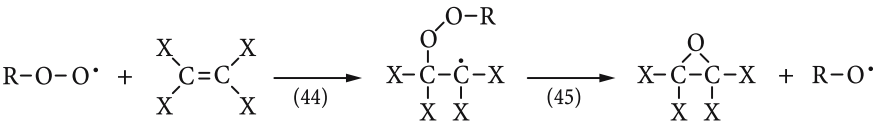
$$\text{R-O-O}^{\bullet} + (\text{CH}_3)_2\text{S} \rightarrow \text{R-O}^{\bullet} + (\text{CH}_3)_2\text{S=O} \qquad (38)$$

In the case of tellurides, strongly oxidizing peroxyl radicals may also undergo ET in competition, and the adduct to phosphines may be sufficiently long-lived to react further with O_2 (Engman et al. 1995).

The trichloromethylperoxyl radical adds to the iodide ion [reaction (39)] with subsequent decomposition into the trichloromethoxyl radical [reaction (40)] which is further reduced by iodide into trichloromethanol [reaction (41); Bonifacic et al. 1991]. Its decay is much faster [reaction (42), $k \geq 8 \times 10^4\ \text{s}^{-1}$] than the subsequent hydrolysis of phosgene [reaction (43), $k = 9\ \text{s}^{-1}$ at 25 °C, $E_a = 53$ kJ mol^{-1}; Mertens et al. 1994].



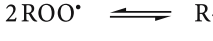
The reaction of peroxyl radicals with alkenes may give rise to epoxides [reactions (44) and (45); cf.; Morgan et al. 1984; Sawaki and Ogata 1984].



The rate constant increases with increasing electron-donating capacity of the C-C double bond (Shoute et al. 1994) and oxidative power of the peroxyl radical (Sawaki and Ogata 1984), and the addition of acylperoxyl radicals has been found to be about 10^5 times faster than that of alkylperoxyl radicals. In the gas phase, the propyl-2-peroxyl radical adds to 2,3-dimethylbut-2-ene with a rate constant of ≈ 6 dm^3 mol^{-1} s^{-1} (Sway and Waddington 1982). With rate constants as low as this, the addition reaction (44) must be the rate determining step of epoxide formation, since the rate of the subsequent step (45) ranges from 10^2 s^{-1} (Phulkar et al. 1990) to 10^6 s^{-1} (Bloodworth et al. 1984).

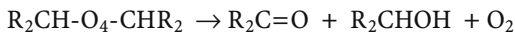
8.8 Bimolecular Decay of Peroxyl Radicals

Peroxyl radicals which do not decay by one of the unimolecular processes discussed above must disappear bimolecularly. In contrast to many other radicals, they cannot undergo disproportionation. Hence they are left to decay via the recombination process, the results of which is a tetroxide intermediate [reaction (46); an exception may be their reaction with $O_2^{\bullet-}$; cf. reaction (7)].,

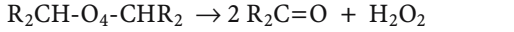


(46)

The tetroxide intermediate is a well-established in organic solvents at low temperatures (Bartlett and Guaraldi 1967; Adamic et al. 1969; Bennett et al. 1970; Howard and Bennett 1972; Howard 1978; Furimsky et al. 1980). However at the temperatures accessible in aqueous solutions the tetroxide, owing to its low ROO-OOR BDE, estimated at 21-33 kJ mol^{-1} (Benson and Shaw 1970; Nangia and Benson 1979; Bennett et al. 1987; Francisco and Williams 1988), can only attain a very low steady-state concentration. Even at the high radical concentrations achievable in the pulse radiolysis experiment, it has not yet been detected. Various decay processes of the tetroxide limits its steady-state concentration: the reverse reaction [reaction (-46)] and its decay into products [reactions (47)–(50), R = alkyl or H]. Most primary (and also some secondary) peroxyl radical decay with rate constants around 10^9 dm^3 mol^{-1} s^{-1} (Neta et al. 1990).



(47)

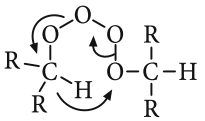


(48)

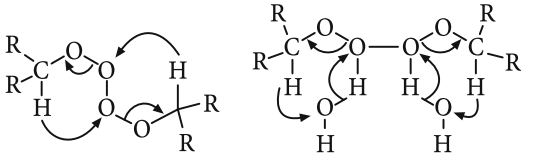
$$R_2CH\text{-}O_4\text{-}CHR_2 \rightarrow 2\,R_2CHO^{\bullet} + O_2 \quad (49)$$

$$R_2CH\text{-}O_4\text{-}CHR_2 \rightarrow R_2CHOOCHR_2 + O_2 \quad (50)$$

Depending on the identity of the peroxyl radicals involved, reactions (47)-(50) may occur in differing proportions. In particular, the product-forming self reaction of tertiary peroxyl radicals is restricted to path (49) and (50), since path (47) and (48) require the existence of C-H α to the peroxyl function. The exact mechanism of these reactions is still controversial, that is, whether the product-forming processes are sequential or concerted. Reaction (47) has been described by Russell (1957) as a concerted process, and this process bears his name. It is formulated as having a six-membered transition state.



Concerted decay "Russell mechanism"



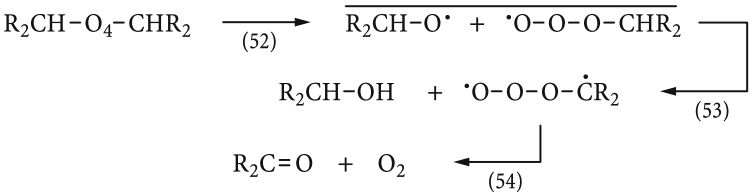
Concerted decay, without and with two water molecules "Bennett mechanism"

Starting from a singlet ground state, the tetroxide gives rise to the carbonyl compound, a hydroxyl compound (usually an alcohol), and O_2, perhaps as singlet dioxygen ($O_2{}^1\Delta_g$) (Nakano et al. 1976; Niu and Mendenhall 1992). Alternatively, O_2 can be formed in its triplet ground state, and the carbonyl compound in its triplet excited state, which is the cause of the chemiluminescence observed in these reactions (Lee and Mendenhall 1988; Mendenhall et al. 1991; Vasvary and Gal 1993).

The concertedness of reaction (47) has been questioned on energetic and several other grounds (Nangia and Benson 1980; Khursan et al. 1990). It has been proposed instead that the carbonyl oxide ($RCH{=}O^{+}\text{-}O^{-}$; the Criegee intermediate), and the alkoxyl radical $R_2CHO^{\bullet}$ play a central role. For aqueous media, this hypothesis which implies a chain reaction (Nangia and Benson 1980) must be ruled out, since on account of the rapid 1,2-H-shift of primary and secondary oxyl radicals under these conditions [reaction (51); Berdnikov et al. 1972; Gilbert et al. 1976, 1977; Schuchmann and von Sonntag 1981] they cannot fulfill their function as chain carrier. Moreover, in the case of the methylperoxyl radical, it could be shown that in aqueous solution the corresponding carbonyloxide may at best play a minor role (Schuchmann and von Sonntag 1984).

$$R_2CH\text{-}O^{\bullet} \rightarrow {}^{\bullet}CR_2OH \quad (51)$$

Asymmetric O-O bond homolysis of the tetroxide as a first step to product formation has been invoked (Khursan et al. 1990), and the idea of the Russell mechanism replaced by a three-step mechanism [reactions (52)–(54)].



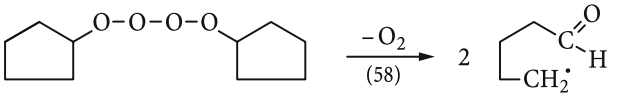
Process (48), sometimes termed Bennett mechanism, yields H_2O_2 and two carbonyl compounds. It has often been thought of as being concerted, proceeding via two five-membered rings (Bennett and Summers 1974; Bothe and Schulte-Frohlinde 1978) or two six-membered rings involving two water molecules (see above). This has been criticized on account of the excessive entropic requirements of the bicyclic transition state, and, following the lateral cleavage (55), reactions (56) and (57) have been proposed instead (Khursan et al. 1990; for a discussion, see von Sonntag and Schuchmann 1997).

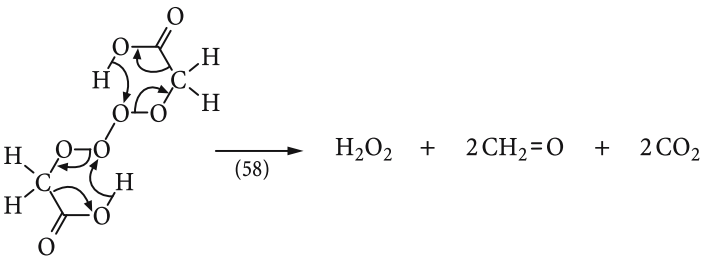
$$R_2CHO^{\bullet} + {}^{\bullet}OOOCHR_2 \rightarrow R_2C{=}O + HOOOCHR_2 \qquad (55)$$

$$HOOOCHR_2 \rightarrow HO_2^{\bullet} + {}^{\bullet}OCHR_2 \qquad (56)$$

$$HO_2^{\bullet} + {}^{\bullet}OCHR_2 \rightarrow H_2O_2 + O{=}CHR_2 \qquad (57)$$

As far as concerted reactions are concerned, it should be mentioned that two other processes of considerable concertedness have been recognized in the studies of the fate of the peroxyl radicals derived from cyclopentane and cyclohexane [e.g., reaction (58); Zegota et al. 1984] and acetate [reaction (59); Schuchmann et al. 1985].





8.9 Alkoxyl Radicals in Peroxyl Radical Systems

In most peroxyl radical systems investigated so-far alkoxyl radicals play a certain, albeit often not dominating role [cf. reaction (49)]. As mentioned above and discussed in more detail in Chap. 7.2, primary and secondary alkoxyl radicals undergo in water rapid ($k \approx 10^6\ s^{-1}$) 1,2-H-shift [reaction (51)]. In competition, β-fragmentation also occurs [reaction (60)].

$$R_3C\text{-}O^\bullet \rightarrow R^\bullet + R_2C{=}O \qquad (60)$$

The rate of this reaction (which is the main decay of tertiary alkoxyl radicals) is also strongly enhanced in water as compared to the gas phase and organic solvents. If different substituents can be cleaved off, it is the more highly-substituted one (weaker C-C bond) that is broken preferentially (Rüchardt 1987). Thus in the case of secondary alkoxyl radicals, substitution in β-position also decides the ratio of 1,2-H-shift and β-fragmentation (Schuchmann and von Sonntag 1982). Because of the fast 1,2-H-shift and β-fragmentation reactions in water, intermolecular H-abstraction reactions of alkoxyl radicals [reaction (61)] are usually inefficient, but intramolecular H-abstraction may occur quite readily if an H atom is in a favorable distance (e.g., six-membered transition state).

$$R_3CO^\bullet + RH \rightarrow R_3COH + R^\bullet \qquad (61)$$

Because of the rapid 1,2-H-shift [reaction (51)] and the ready conversion of the ensuing α-hydroxyalkyl radical into $HO_2^\bullet/O_2^{\bullet-}$ by O_2 [cf. reactions (8) and (10)/(11)], primary and secondary peroxyl are often the precursor of $O_2^{\bullet-}$ in theses systems. Furthermore, the β-fragmentation reaction (60) creates a new radical and hence a new peroxyl radical, a situation which makes the elucidation of mechanistic details often very difficult if not impossible. The peroxyl radical systems that have been investigated in detail thus far have been discussed by von Sonntag and Schuchmann (1997).

8.10 Oxygen Uptake

Oxygen uptake measurements can give considerable mechanistic information. In normal peroxyl radical reactions the $G(O_2\text{-uptake})$ will range between 3×10^{-7} and 6×10^{-7} mol J^{-1} (Table 8.2). The lower value will be found when half of the O_2 is reformed (for example, the formate system), the higher value when all O_2 is consumed during the decay of the peroxyl radicals. As soon as $G(O_2\text{-uptake})$ exceeds the upper limit of 6×10^{-7} mol J^{-1}, a chain reaction must prevail [examples are some polymers including poly(U)]. However, there is also the interesting situation that $G(O_2\text{-uptake})$ is below 3×10^{-7} mol J^{-1}. This means that some of the radicals do not react with O_2. A case in point are the $^\bullet OH$-induced reactions of purines (Chap. 10.3).

Table 8.2. *G* values (unit: 10^{-7} mol J^{-1}) of O_2-uptake in the γ-radiolysis of several substrates in N_2O/O_2-saturated solutions. For substrate concentrations and dose rates see the original literature

Substrate	•OH	Tl(II)	Reference
Formate	3.1 3.1		Isildar et al. (1982) Al-Sheikhly (1994)
2-PrOH	3.1		Isildar et al. (1982)
*t*BuOH	4.5		Isildar et al. (1982)
Diethyl ether	4.7		Isildar et al. (1982)
Carbowax 20 M	10.6		Isildar et al. (1982)
Poly(acrylic acid), pH 10	100		Ulanski et al. (1996a)
Poly(methyl vinyl ether)	110		Janik et al. (2000)
2-Deoxyribose	3.1 3.1	 0.77	Isildar et al. (1982) Al-Sheikhly (1994)
Thy	4.2		Isildar et al. (1982)
Cyt	4.6		Isildar et al. (1982)
Ura	5.2		Isildar et al. (1982)
Thd	5.0		Isildar et al. (1982)
dAdo	1.4 1.75	0.72	Isildar et al. (1982) Al-Sheikhly (1994)
Ade	2.7	0.82	Al-Sheikhly (1994)
dGuo	1.6		Isildar et al. (1982)
dGuo	1.5	0.72	Al-Sheikhly (1994)
Guo	1.5	0.72	Al-Sheikhly (1994)
TMP	4.6		Isildar et al. (1982)
dCMP	5.0		Isildar et al. (1982)
dAMP	2.0		Isildar et al. (1982)
dGMP	1.7		Isildar et al. (1982)
UMP	6.6		Isildar et al. (1982)
poly(U)	21.8		Isildar et al. (1982)
poly(A)	3.6		Isildar et al. (1982)
ssRNA	4.2		Isildar et al. (1982)
ssDNA	7.1		Isildar et al. (1982)
Mixture of 2′-deoxynucleotides as in DNA	3.3		Isildar et al. (1982)
Ditto calculated from the above data	3.3		Isildar et al. (1982)

8.11 The Superoxide Radical

In the preceding paragraphs, various routes to $HO_2^{\bullet}/O_2^{\bullet-}$ upon unimolecular and bimolecular decay of peroxyl radicals have been shown. Although these reactions will certainly contribute, in a living cell the main source of $O_2^{\bullet-}$ is a side reaction in the energy-providing mitochondrial metabolism (Turrens 1997; for an estimate of cellular steady-state concentrations see Boveris and Cadenas 1997). In a human, its production has been estimated at 0.2-0.4 mol/day. Especially, when cells residing in a hypoxic condition are exposed to O_2, large quantities of $O_2^{\bullet-}$ are set free, an effect that contributes to, if it is not the cause of, ischemia. Moreover, $O_2^{\bullet-}$ is produced in large quantities by macrophages as a part of their defense system. Thus, $O_2^{\bullet-}$ is the most ubiquitous peroxyl radical. In a cellular environment it must exert severe damage (Fridovich 1978), and it is generally considered to be a major player in what is termed as "oxidative stress" (Sies 1986, 1991; for the repair of oxidative damage in mitochondrial DNA see Bohr 2002). Evolution produced enzymes, the superoxide dismutases (SOD), to keep cellular $O_2^{\bullet-}$ steady-state concentration low (for reviews see Fridovich 1975; Oberley 1982).

There are three types of SOD. All SODs have transition metal ions in their reaction center, and the enzymes in both high and low oxidation states react readily ($k \approx 2 \times 10^9$ dm^3 mol^{-1} s^{-1}; Klug et al. 1972; Fielden et al. 1974; Pick et al. 1974) with $O_2^{\bullet-}$ [written with Cu as an example; reactions (62) and (63)] which allows the enzyme to be recycled.

$$SOD(Cu^{2+}) + O_2^{\bullet-} \rightarrow SOD(Cu^{+}) + O_2 \qquad (62)$$

$$SOD(Cu^{+}) + O_2^{\bullet-} + 2\,H^{+} \rightarrow SOD(Cu^{2+}) + H_2O_2 \qquad (63)$$

The one isolated from bovine blood contains Cu in its reaction center as well as Zn which appears not to take part in the dismutation process. The other two types of SOD contain either Fe or Mn. The CuZn SOD has been found only in eukariotic cells, the Fe SOD only in prokaryotic cells, and the Mn SOD in both (Fee 1981). Aqua-Mn^{2+} cannot be reduced by $O_2^{\bullet-}$, but is forms a complex that dismutates giving rise to H_2O_2 and O_2 (Jacobsen et al. 1997). Such intermediates may also play a role in Mn SOD.

For the study of its reaction in aqueous solutions, $O_2^{\bullet-}/HO_2^{\bullet}$ may be generated by various means (for a review see Cabelli 1997). One possibility is the vacuum-UV photolysis of aqueous solutions of EtOH at high pH (Bielski and Gebicki 1982; for a review on VUV photolysis of alcohols, see von Sonntag and Schuchmann 1977). Under these conditions, α-hydroxyalkyl is generated which, as discussed above, is readily converted by O_2 to $O_2^{\bullet-}$, which is long-lived at high pH (see below). This method of generating $O_2^{\bullet-}$ has the advantage that besides EtOH and some acetaldehyde no further potentially reactive material is present in these $O_2^{\bullet-}$ solutions. For the photolytic generation of $O_2^{\bullet-}$ at longer wavelengths, the reaction of $n\rightarrow\pi^*$ excited benzophenone with alcohols in aqueous solution can be used as well (McDowell et al. 1983). Alternatively, it also can be generated

radiolytically in O_2-containing aqueous formate solution (Bielski and Richter 1977). Dissolving KO_2 in DMSO also affords $O_2^{\bullet-}$, and in a stopped-flow system this solution may be diluted with water, allowing the study of $O_2^{\bullet-}$ at least at a moderately alkaline pH (Bull et al. 1983). The thermal decomposition of the azo compound di(4-caboxybenzoyl)hyponitrite (SOTS-1) also yields $O_2^{\bullet-}$ (Ingold et al. 1997; Chap. 2.4). Most commonly, $O_2^{\bullet-}$ is generated enzymatically by xanthine/xanthine oxidase. Thereby, the reduced xanthine oxidase reacts with O_2 to produce $O_2^{\bullet-}$ via a stepwise mechanism (Fridovich 1970), and altogether two $O_2^{\bullet-}$ radicals are generated for each xanthine oxidized. A caveat has been expressed (Ingold et al. 1997) that the enzyme may contain an excess of transition metal ions which can lead to the formation of $^{\bullet}OH$ via the Fenton reaction, which then can suggest a reactivity of $O_2^{\bullet-}$, which in fact does not exist. The fact that $O_2^{\bullet-}$ is formed in some autoxidation reactions has been connected with the cytotoxic properties of such compounds (Cohen and Heikkila 1974).

In neutral solution, $O_2^{\bullet-}$ dominates. The $pK_a(HO_2^{\bullet}) = 4.8$ is the selected value (Bielski et al. 1985) from a series of determinations (Czapski and Bielski 1963; Czapski and Dorfman 1964; Sehested et al. 1968; Rabani and Nielsen 1969; Behar et al. 1970). The rate of self-termination of $HO_2^{\bullet}/O_2^{\bullet-}$ strongly depends on pH, since only reactions (64) and (65) proceed at an appreciable rate ($k_{64} = 8.6 \times 10^5$ dm^3 mol^{-1} s^{-1} ; $k_{65} = 1.02 \times 10^8$ dm^3 mol^{-1} s^{-1}), while the self-termination of two $O_2^{\bullet-}$ is too slow to be measurable ($k < 0.35$ dm^3 mol^{-1} s^{-1}; Bielski et al. 1985).

$$2\,HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \qquad (64)$$

$$HO_2^{\bullet} + O_2^{\bullet-} \rightarrow HO_2^{-} + O_2 \qquad (65)$$

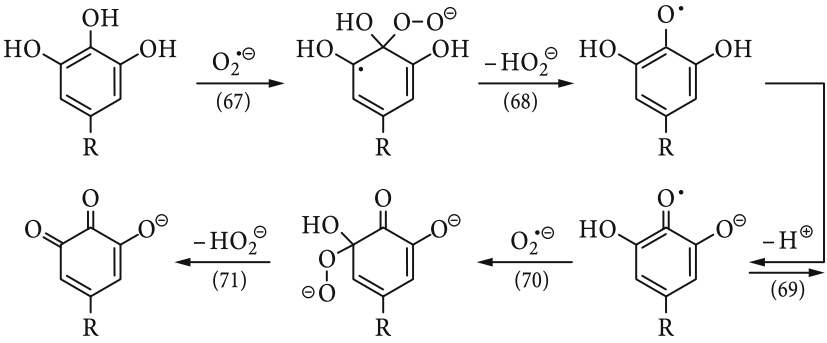
As a consequence, the observed rate of bimolecular decay has a maximum at the pK_a value, levels of at low pH at the rate constant of reaction (64), and at high pH continues to drop when $\log k_{obs}$ is plotted vs. the pH. In acid solution, the activation energy of the bimolecular decay of $HO_2^{\bullet}$ is 24 kJ mol^{-1} (Bielski and Saito 1962), in D_2O it rises to 29 kJ mol^{-1} (Bielski and Saito 1971). There is increasing evidence that little or no (<10%) singlet dioxygen ($O_2{}^1\Delta_g$) is formed upon the bimolecular decay of $HO_2^{\bullet}$ (Foote et al. 1980; Aubry et al. 1981; Arudi et al. 1984).

The detection of $O_2^{\bullet-}$ is usually based on its reducing properties ($E^7 = -0.33$ V; Ilan et al. 1974; Wardman 1989). A typical example is its reaction with TNM [reaction (66); $k = 1.9 \times 10^9$ dm^3 mol^{-1} s^{-1}; Asmus and Henglein 1964; Rabani et al. 1965] which yield the strongly absorbing nitroform anion ($\varepsilon(350\text{ nm}) = 15{,}000$ dm^3 mol^{-1} cm^{-1}].

$$O_2^{\bullet-} + C(NO_2)_4 \rightarrow O_2 + C(NO_2)_3^{-} + {}^{\bullet}NO_2 \qquad (66)$$

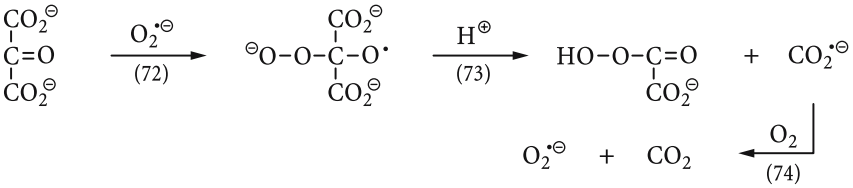
Similarly, nitro blue tetrazolium is reduced by $O_2^{\bullet-}$ ($k = 3 \times 10^4$ dm^3 mol^{-1} s^{-1}), and the mono-reduced species subsequently disproportionates yielding the two-electron-reduced monoformazan which absorbs in the visible ($\varepsilon(530) = 2.34 \times 10^3$ dm^3 mol^{-1} cm^{-1}; Bielski et al. 1980; for some of the problems that one may encounter using this assay see Cabelli 1997].

Except for ET reactions with strong oxidants, $O_2^{\bullet-}$ is not very reactive (for a compilation of rate constants, see Bielski et al. 1985). For example, practically no reaction has been detected with amino acids (Bielski and Shiue 1979), and there is no reaction to speak of with the DNA constituents, that is, it is also practically unreactive towards DNA. However, where substantial reactivity has been recognized, its main route of reaction seems to be by addition. This has not only been proposed for its reaction with pyrogallol and the propyl ester of gallic acid [$k = 3.4 \times 10^5$ and 2.6×10^5 dm^3 mol^{-1} s^{-1}, respectively; cf. reactions (67)–(71); Deeble et al. 1987, 1988], but it seems that an addition reaction triggers a number of chain reactions (von Sonntag et al. 1993, see below).



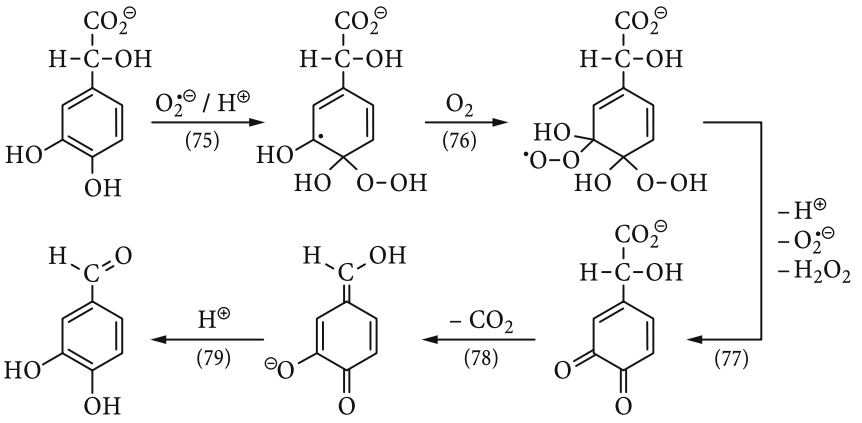
Interestingly, the reactivity of the (more oxidizing) $HO_2^{\bullet}$ radical is much less than that of $O_2^{\bullet-}$, although as a net reaction an oxidation has occurred. This has been attributed to kinetic reasons, that is, $HO_2^{\bullet}$ does not undergo addition reactions as readily as $O_2^{\bullet-}$.

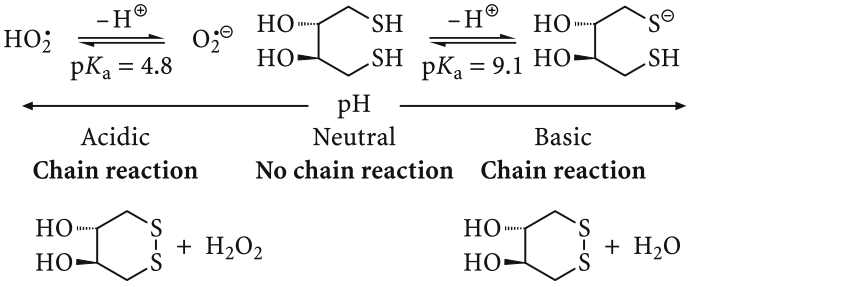
The reaction of $O_2^{\bullet-}$ with ketomalonic acid leads to oxalic peracid and CO_2 [Schuchmann et al. 1991; reactions (72)–(74)].



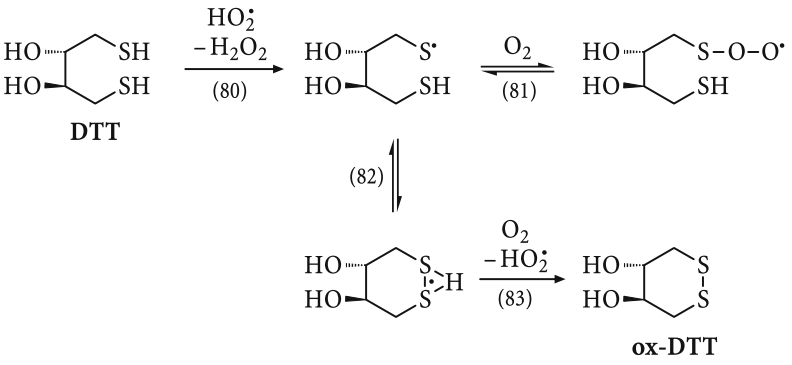
Decarboxylation is also observed in its reaction with 3,4-dihydroxymandleic acid [reactions (75)–(79); Deeble and von Sonntag 1992].

With 1,4-dithiotreitol (DTT), $O_2^{\bullet-}$ undergoes a chain reaction in basic solution. This reaction leads to 1,4-*threo*-dihydroxy-1,2-dithian (ox-DTT) and water (Zhang et al. 1991). However, when DTT is no longer deprotonated as in neutral solution, this chain reaction ceases. In acid solutions, where the $HO_2^{\bullet}$ predominates, another chain reaction takes place. It now leads to ox-DTT and H_2O_2 (Lal et al. 1997).

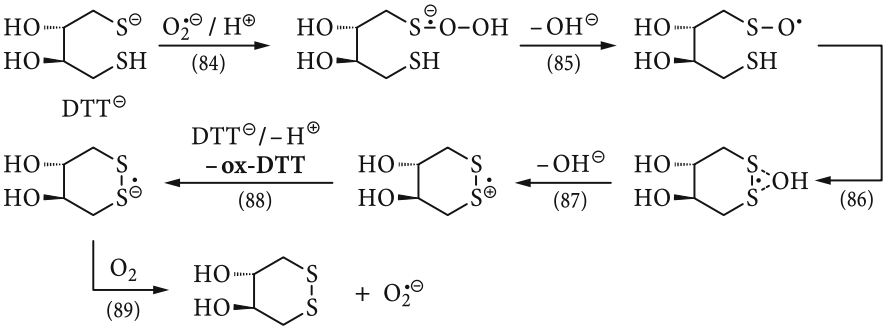




Thus, $O_2^{\bullet-}$ and $HO_2^{\bullet}$ radicals behave very differently. While the $HO_2^{\bullet}$ radical undergoes an H-abstraction [reactions (80)–(83)] and behaves like an ordinary peroxyl radical, the $O_2^{\bullet-}$ reaction sequence is initiated by an addition reaction [reaction (84), see below]. The H-abstraction reaction (80) is slow (k = 120 dm^3 mol^{-1} s^{-1}). Similar conclusions, revising an earlier report (Schulte-Frohlinde et al. 1986), have been obtained by Hildenbrand and Schulte-Frohlinde (1997) for the reaction of DNA peroxyl radicals with GSH.

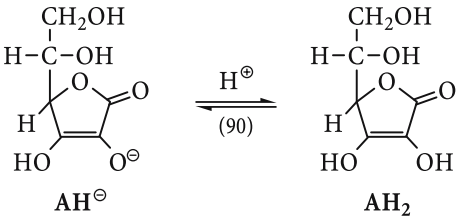


The mechanistic proposal for the chain reaction of $O_2^{\bullet-}$ with the DTT anion represented by reactions (84)–(89) (Lal et al. 1997) deviates slightly from the original proposal (Zhang et al. 1991). The essential aspect, however, remains the addition of $O_2^{\bullet-}$ to the thiolate thereby forming a three-electron-bonded intermediate [reaction (84); for other three-bonded intermediates see Chap. 7.4] and its subsequent decay into an oxidizing species [reaction (85)]. It has been calculated that the rate constant of the rate-determining step, reaction (84), is 35 dm^3 mol^{-1} s^{-1}. This reaction is even slower than the H-abstraction reaction of the $HO_2^{\bullet}$ radical discussed above.



The reactivity of a wider range of thiols toward $O_2^{\bullet-}$ has been studied (Winterbourn and Metodiewa 1999), and it has been observed that at pH 7.4 only those thiols show an appreciable reactivity who have a low pK_a value, such as penicillamine and cysteine (at pH 7, the rate constant of $O_2^{\bullet-}$ with *N*-actylcysteine is 68 dm^3 mol^{-1} s^{-1}; Benrahmoune et al. 2000). This can be rationalized by assuming that a reasonable thiolate steady-state concentration is required for the reaction to proceed. In the case of GSH the disulfide was again the major product, but the formation of some glutathionesulfonic acid was also reported (Winterbourn and Metodiewa 1994). In the biological context, the reactivity of $O_2^{\bullet-}$ with GSH is of major importance. Various rate constants are reported in the literature; the most recent re-evaluation gives now a value of ~200 dm^3 mol^{-1} s^{-1}, indicating that in a cellular environment this reaction is too slow to compete with the elimination of $O_2^{\bullet-}$ by SOD (Jones et al. 2002, 2003).

In the cellular environment, ascorbic acid (AH_2) plays a major role. Its pK_a value is at 4.3 [equilibrium (90)], and hence the ascorbate anion (AH^-) predominates around neutrality.



Both $HO_2^•$ and $O_2^{•-}$ react with ascorbate [reactions (91)–(94); Nishikimi 1975; Cabelli and Bielski 1983].

$$AH_2 + HO_2^• \rightarrow A^{•-} + H_2O_2 + H^+ \quad (91)$$

$$AH_2 + O_2^{•-} \rightarrow A^{•-} + H_2O_2 \quad (92)$$

$$AH^- + HO_2^• \rightarrow A^{•-} + H_2O_2 \quad (93)$$

$$AH^- + O_2^{•-} \rightarrow \text{product(s)} \quad (94)$$

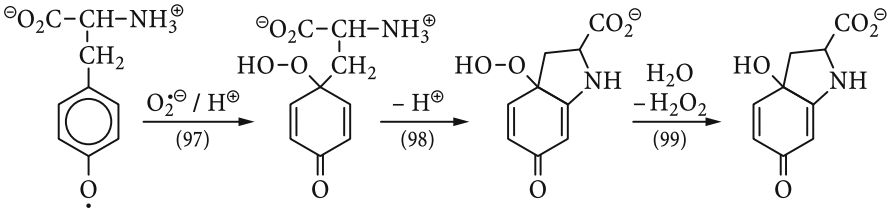
The value for k_{91} is 1.4×10^4 dm^3 mol^{-1} s^{-1}, and that for k_{94} is 5×10^4 dm^3 mol^{-1} s^{-1}. Kinetically, reactions (92) and (93) cannot be separated ($k_{93} + 0.36\ k_{92} = 1.2 \times 10^7$ dm^3 mol^{-1} s^{-1}).

The ascorbate radicals also react very rapidly with $O_2^{•-}/HO_2^•$ [reactions (95) and (96), $k_{95} = 5 \times 10^9$ dm^3 mol^{-1} s^{-1}, $k_{96} = 2.6 \times 10^8$ dm^3 mol^{-1} s^{-1}].

$$A^{•-} + HO_2^• \rightarrow \text{products} \quad (95)$$

$$A^{•-} + O_2^{•-} \rightarrow \text{products} \quad (96)$$

The ascorbate radical is one of the radicals that do not react readily with O_2, but it reacts with $O_2^{•-}$. The product of this reaction is not yet known. There are other radicals that have similar properties such as phenoxyl-type radicals. A prominent member of this group is the vitamin E radical. In the phenoxyl radical series, addition as well as ET have been discussed (Jonsson et al. 1993; d'Alessandro et al. 2000). The reaction of the tyrosyl radical with $O_2^{•-}$ is an example showing that addition is the main route despite of its relatively high redox potential [reactions (97)–(99); only one pathway is shown; Jin et al. 1993].



The *N*-centered tryptophan (Fang et al. 1998) and bisbenzimidazole radicals (Adhikary et al. 2000) also do not react with O_2, but readily with $O_2^{•-}$, the former by addition. In the nucleobase series, a similar situation prevails for G$^•$ (k(G$^•$ + $O_2^{•-}$) = 4.4×10^9 dm^3 mol^{-1} s^{-1}; von Sonntag 1994). Most of the reaction seems to occur by ET reforming G plus O_2, but in competition about 15% to lead to products such as Iz and 8-oxo-G (Misiaszek et al. 2004).

The $O_2^{•-}/HO_2^•$ radicals can be trapped by spin traps, but the rate constants are low and their lifetime is often only short (Lauricella et al. 2004), i.e., they give rise to the $^•$OH-adduct radical upon hydrolysis (Chap. 3.4). To increase the

lifetime of the DEPMPO adduct, it has been suggested to freeze the solution to liquid nitrogen temperature for detection (Dambrova et al. 2000).

8.12 The 'Haber-Weiss Reaction'

In their brilliant papers, Haber and Weiss (1932, 1934) studied the iron-catalyzed decomposition of H_2O_2 and proposed the reaction of $HO_2^{\bullet}$ with H_2O_2 as a single step [reaction (100)]. This has since been shown to proceed in two steps [reactions (101) and (102); Czapski and Ilan 1978; Bielski 1985; Rush and Bielski 1985].

$$HO_2^{\bullet} + H_2O_2 \rightarrow H_2O + O_2 + {}^{\bullet}OH \quad (100)$$

$$HO_2^{\bullet} + Fe^{3+} \rightarrow O_2 + Fe^{2+} + H^+ \quad (101)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (102)$$

The latter is well known as 'Fenton reaction' (Fenton and Jackson 1899). Over the years, the 'Haber-Weiss reaction' [reaction (100)] has often been assumed to be the source of $^{\bullet}OH$. The much greater likelihood of transition metal catalysis, which is also possible in biological systems, has often been overlooked. Traces of transition metal ions may be present in otherwise pure model systems (Fridovich and Porter 1981), and may even be leached out of the glassware (Ulanski et al. 1996c). It is now clear that the uncatalyzed reaction (100) is slow [$k = 0.2$ dm^3 mol^{-1} s^{-1}; Ferradini and Seide 1969; $k = 0.5$ dm^3 mol^{-1} s^{-1}, $k(O_2^{\bullet-} + H_2O_2) = 0.13 \times 10^9$ dm^3 mol^{-1} s^{-1}; Weinstein and Bielski 1979] and can be neglected for the majority of experimental conditions. The thermodynamics of the uncatalyzed and catalyzed 'Haber-Weiss reaction' has been discussed in some detail by Koppenol (1983).

In contrast to the Haber-Weiss reaction, the reaction of $O_2^{\bullet-}$ with HOCl [reaction (103)] proceeds without transition metal catalysis (Candeias et al. 1993).

$$HOCl + O_2^{\bullet-} \rightarrow {}^{\bullet}OH + Cl^- + O_2 \quad (103)$$

This reaction is considered to be an important step in the phagocytic killing of microorganisms by free-radical processes (Saran et al. 1999). In this reaction, also $Cl^{\bullet}$ was considered to play a role (for the complexities of $^{\bullet}OH/Cl^-$ reactions in aqueous solution, see Yu and Barker 2003a,b; Yu et al. 2004).

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Polymer Radicals

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9.1 General Remarks

Linear man-made polymers and also some biopolymers may have only one repeating unit, but often there are more than one repeating unit which do not follow one another in a regular way. With its four nucleotides as repeating units, DNA is a typical example. In solution, neutral linear polymers attain a coil-like conformation. In charged polymers, the charges repel each other, and the conformation of the polymer becomes rod-like. The local density of the repeating units and the conformation of the polymer chain have a dramatic effect on some of the properties of the free-radical chemistry of the polymer (Ulanski et al. 1997). Quite a number of the properties of DNA radicals can be understood if it is taken into account that DNA is a highly charged polymer.

9.2 Rate of Formation of Polymer Radicals

The rate of reaction of neutral free radicals such as $^{\bullet}OH$ with a polymer or an equal concentration (in g dm^{-3}) of its subunits is generally much lower for the polymer than that of the low molecular weight compound. The low molecular weight molecules are randomly distributed throughout the solution, while the polymer chains do not fill equally well the whole space. Thus, the average distance that a reactive free-radical has to travel to reach the target molecule is much longer in the case of the polymer. Experimentally, this has been shown with many synthetic polymers (Behzadi et al. 1970; Ulanski et al. 1995), and the rate of reaction of DNA with $^{\bullet}OH$ is also much lower (2.5×10^8 dm^3 mol^{-1} s^{-1}; Udovicic et al. 1994; for corresponding data on polynucleotides see Michaels and Hunt 1973) than that with nucleotides (~3×10^9 dm^3 mol^{-1} s^{-1}; for a compilation see Chap. 10.3). The rate constants of $^{\bullet}OH$ are often determined in competition with SCN^-. With charged polymers such as DNA or poly(A) (Loman and Ebert 1968), this may lead to complications and eventually to erroneous results due to a repulsion of SCN^- from the highly charged polymer (Ulanski and von Sonntag 2000).

The rate of reaction of e_{aq}^- with charged polymers such as polynucleotides is much lower than that of parent bases (Shragge et al. 1971). An electrostatic repulsion of e_{aq}^- by the negatively charged polymer adds to the effect discussed above. These much lower rates of polymers as compared to their subunits also allows one to determine the binding constants of drugs, e.g., ethidium bromide to DNA (Greenstock et al. 1977).

Competitive scavenging of reactive free radicals, such as $^{\bullet}OH$, by DNA and other substrates, is a very important aspect of DNA free-radical chemistry. This situation has been modeled by various approaches (van Rijn et al. 1985; Lafleur and Loman 1986; Verberne et al. 1987). The most recent model has been developed by Udovicic et al. (1991).

Increasingly, Monte-Carlo calculations become available, which try not only to describe the general rate of reactions but also the sites of attack within the

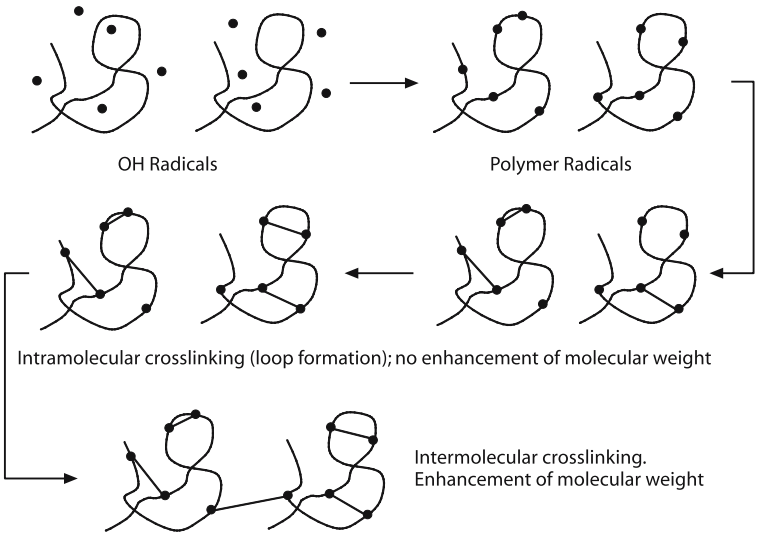


Fig. 9.1. Schematic description of the •OH-induced formation of polymer radicals and their subsequent decay

DNA (Chatterjee and Magee 1985; Wright et al. 1985; Valota et al. 2003). Chapter 12.2 refers to these studies.

9.3 Lifetime of Polymer Radicals

In general, radicals have to terminate bimolecularly. Polymer radicals may react *intra*molecularly with another radical situated at the same polymer molecule or *inter*molecularly with a radical at another polymer molecule. With a large number of radicals at the same macromolecule, such as are produced at the high dose rate of pulse radiolysis and at low polymer concentrations, the former process is usually favored (Fig. 9.1).

The kinetics of the bimolecular decay of poly(vinyl alcohol) (Ulanski et al. 1994) and poly(vinyl methyl ether) radicals (Janik et al. 2000b) have been studied in some detail (cf. Fig. 9.2). The •OH radicals formed during the pulse generate on the (coil-shaped) polymer a non-random distribution of radicals. First, the radicals which are very close to one another recombine. The intrinsic bimolecular rate constant for such a process can be much faster than that of the decay of an equal concentration of randomly distributed low molecular weight radicals. As the number of close-by radicals decreases, the intrinsic rate constant drops, and the lifetime of the polymer radicals increases considerably. Now, the bimolecular decay of the polymer radicals becomes much slower than that of the corresponding low molecular weight radicals. While in the case of low molecular weight radicals the bimolecular rate constant is independent of the

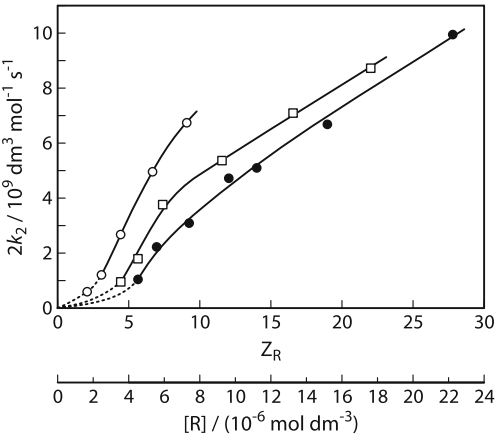


Fig. 9.2. Pulse radiolysis of poly(vinyl alcohol) (10^{-3} mol dm^{-3}) in N_2O-saturated aqueous solutions at pH 5.5. Incremental second-order rate constants as a function of a given radical concentration ([R]; *upper scale*) or radicals per macromolecule (Z_R; *lower scale*). *Open circles* 20 Gy; *open squares* 65 Gy; *filled circles* 120 Gy. Source: Ulanski et al. (1994), with permission

radical concentration, in polymer radicals it strongly depends on the number of radicals per polymer molecule (for a theoretical treatment of this phenomenon see Raap and Gröllmann 1983). With only a few radicals on the polymer chain, the lifetime of the radicals can become considerable, and due to the slowness of the motion of macromolecules, intermolecular reactions with radicals situated at other polymers is usually a rare event.

When the polymer is charged, the repulsive forces of the charges prevent an approach of the radicals, and the lifetime of the radicals increases dramatically. In the case of poly(acrylic acid), for example, the decay of the poly(acrylic) acid radicals is fast and follows the same kinetics as any radical derived from neutral polymers as long as the polymer is fully protonated (low pH) (Ulanski et al. 1996c). With increasing pH and concomitant dissociation of the polymer, however, the polymer assumes a rod-like shape, its segments become less flexible, and repulsive forces increasingly prevent their approach. Some radicals survive even for hours under such conditions.

Interestingly, the corresponding peroxyl radicals have a noticeably shorter lifetime (Ulanski et al. 1996c). This has been explained by the fact that these radicals do not have to approach one another as closely, and thus the repulsive forces are less effective (Ulanski et al. 1997).

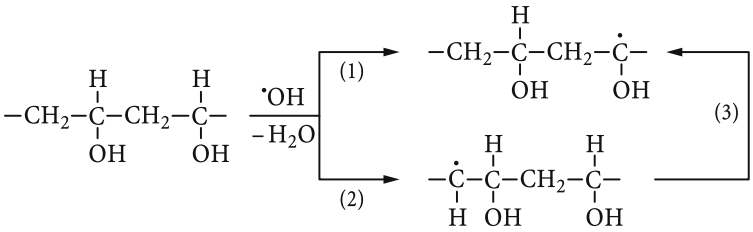
9.4 Radical Transfer

A polymer usually has more than one functional group, more than one type of radical is formed upon •OH-attack. These radicals may undergo radical transfer

either by H-abstraction from a position where the H-atom is more loosely bound, or add to double bonds. The polymer radical experiences a very high local concentration of these functional groups as long as the polymer chains are flexible, and the rate of this (*intra*molecular) reaction will be much higher than an equivalent *inter*molecular reaction of low molecular weight models at the same concentration. In a charged polymer, the rate of this process decreases with increasing charge of the polymer due to a lower flexibility of segments. On the other hand, since this process is kinetically of first order while radical recombination processes are of second order, such a process may only become prominent when the polymer is highly charged, that is, when the bimolecular termination becomes slow. Two competing processes are discussed here, H-transfer and β-fragmentation. In DNA, there is also the possibility that radicals may add intramolecularly to a double bond at one of the bases. Evidence for such a reaction is not yet on firm ground, but this possibility should be kept in mind.

9.4.1 Intramolecular H-Transfer

In poly(vinyl alcohol), •OH abstracts preferentially a hydrogen in α-position to the hydroxyl group (forming the tertiary radical, 70%) but also from the methylene group (forming the secondary radical, 30%) [reactions (1) and (2)]. The C-H BDE of the secondary hydrogen is somewhat higher than that of the tertiary hydrogen in the -CHOH- group, and thus the secondary radicals undergo the H-abstraction reaction (3) (k_3 = 460 s^{-1}; von Sonntag et al. 1999; see also Chap. 6.5). Whether the reaction occurs with the first available hydrogen [five-membered



transition state as depicted in reaction (3)] or over a larger distance, is as yet unknown. The rate of this H-transfer is only that fast, because it occurs as an *intra*molecular process. In order to compare this first-order rate with known second-order processes, one may take the concentration of neighboring -CHOH- groups in the polymer as 10 mol dm^{-3}, that is, the second-order rate constant should not exceed 50 dm^3 mol^{-1} s^{-1} by much. For comparison, the somewhat more exothermic H-transfer from 2-PrOH to the •CH_2-CHOH-CH_3 radical [H-abstraction by a primary alkyl radical as compared to a secondary one as in reaction (3)] has a rate constant of 430 dm^3 mol^{-1} s^{-1} (Burchill and Wollner 1972).

In poly(acrylic acid), two radicals are also formed upon •OH-attack. Again, the secondary radical undergoes *intra*molecular H-abstraction, leading to the tertiary radical [reaction (4); Ulanski et al. 1996c].

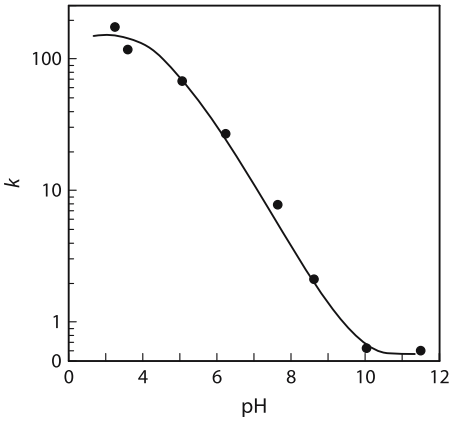
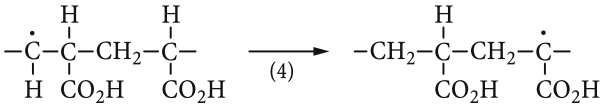
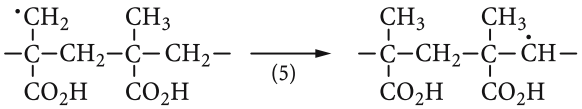


Fig. 9.3. Pulse radiolysis of N_2O-saturated solutions of poly(acrylic acid). First-order rate constant of the β- to α-radical conversion as a function of pH. Source: Ulanski et al. (1996c), with permission

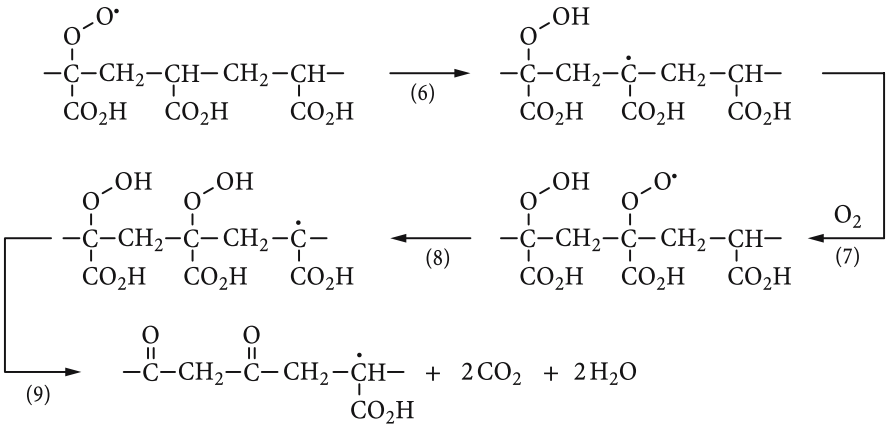


The rate of this reaction strongly depends on the pH (Fig. 9.3), i.e., the protonation state of the polymer. With increasing pH, the rate of reaction slows down considerably, an indication that the flexibility of the polymer chain is of importance for this reaction to occur efficiently. Whether this implies that H-abstraction mainly occurs from distant sites and not from a neighboring subunit [as shown in reaction (4)], cannot be decided yet on the basis of the existing data.

A similar H-abstraction takes place in poly(methacrylic) acid, converting the primary radical into a secondary one [reaction (5); $k = 350\ s^{-1}$ at pH 7.5; Ulanski et al. 1999b].

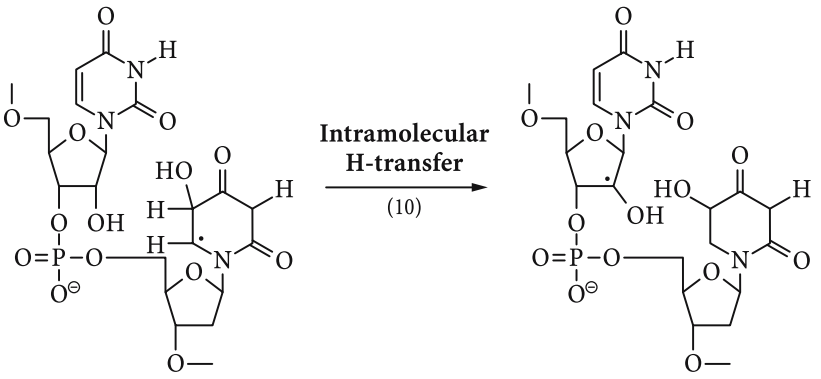


When in the poly(acrylic acid) system radical the tertiary radical is converted by O_2 into the corresponding peroxyl radical, a chain reaction sets in which yields CO_2 and a acetylacetone-like product [reactions (6)–(9); Ulanski et al. 1996a; for the formation of acetylacetone in the model system 2,4-dimethylglutaric acid, see Ulanski et al. 1996b].



The CO_2 yield considerably exceeds that of the acetylacetone-like product, and although CO_2 is certainly also formed in other reactions (cf. the low molecular weight model; Ulanski et al. 1996b), this observation may be taken as a hint that the H-abstraction reaction does not proceed with neighboring groups only (via a favorable six-membered transition state) but also with more distant sites. A chain-type autoxidation has also been observed with poly(vinyl methyl ether) and a related model compound (Janik et al. 2000a,b).

A polymer much closer to DNA is poly(U). With this polymer, base radicals and base peroxyl radicals abstract an H-atom sugar moiety of the neighboring or a close-by nucleotide [e.g., reaction (10); Deeble and von Sonntag 1984, 1986; Deeble et al. 1986].



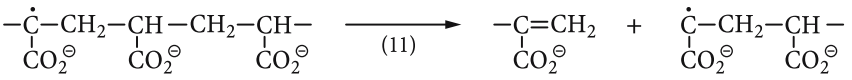
Compared to DNA, this H-transfer to the sugar moiety is especially favored in poly(U) or poly(C) (Chap. 11.2) by the low C(2′)-H BDE induced by the OH group in α-position (Chap. 6.5).

9.4.2
Intermolecular H-Transfer

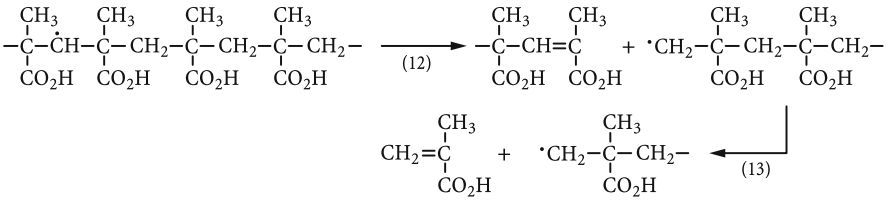
In charged polymers, a strong influence of charge on the rate of H-abstraction is also observed with external thiols as hydrogen donors. A case in point is poly(U) (Fahey et al. 1991). As mentioned above, base-centered radicals can abstract an H atom from the sugar moiety, and the resulting radical undergoes chain scission (see below). This reaction can be prevented by thiols which donate an H-atom to the base radical. Since poly(U) is a charged polymer, the rate of H-transfer to the polymer strongly depends on the charge of the thiol (Chap. 11.2).

9.4.3
Homolytic β-Fragmentation and Hydrolysis

Homolytic β-fragmentation is a common process in free-radical chemistry. It is the reverse of the better known polymerization. Although β-fragmentation is often rather slow and has to compete with the bimolecular termination reaction of free radicals, it can become of considerable importance in long-lived radicals such as the poly(acrylic acid) radicals (charged at high pH), where this reaction leads to chain scission [reaction (11); von Sonntag et al. 1995; Ulanski et al. 1996c].

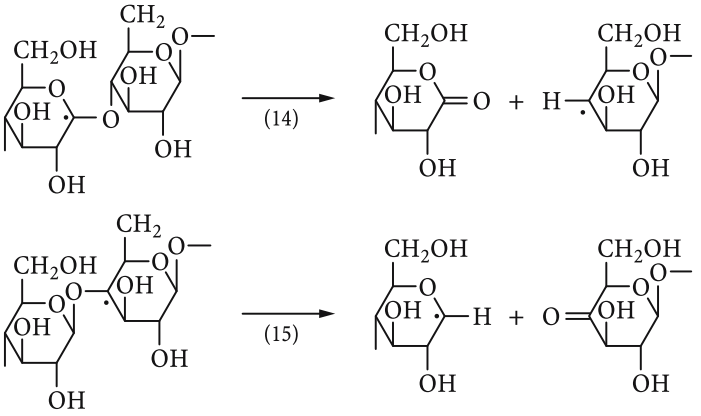


In poly(methacrylic acid), where this type of reaction is ~50 times faster (von Sonntag et al. 1995; Ulanski et al. 1999a), even at room temperature subsequent depolymerization occurs as a very efficient process [reactions (12) and (13); Ulanski et al. 1999a,b].

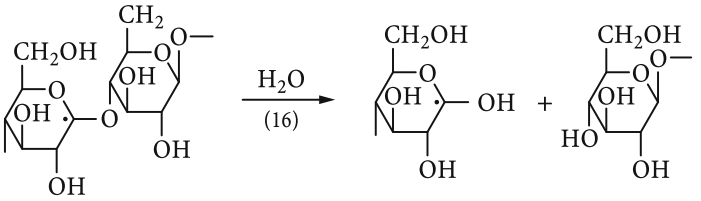


Because of a ready addition of the methacrylic acid released in reaction (13) to all radicals in this system, the situation of equilibrium polymerization is approached even at room temperature (Ulanski et al. 2000).

In carbohydrates, β-fragmentation of radicals adjacent to the glycosidic linkage [e.g., reactions (14) and (15)] will also lead to chain scission.



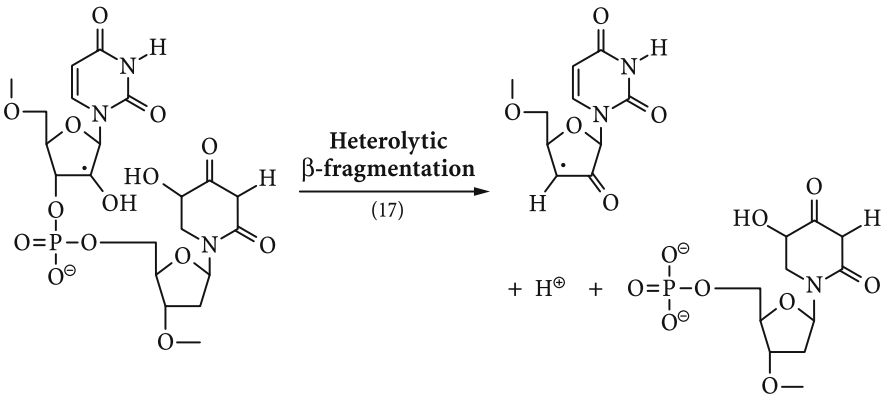
These reactions are in competition with a fast hydrolysis of the glycosidic linkage [e.g. reaction (16)] which can be many orders of magnitude faster than that of the parent.



These reactions are that fast that they occur at the time scale of pulse radiolysis (Deeble et al. 1990, 1991; Ulanski and von Sonntag 2000). In DNA, such reactions will not lead immediately to a stand break, but they may well contribute to base release.

9.4.4 Heterolytic β-Fragmentation

Radicals can also eliminate an anion in β-position thereby forming a radical cation. This reaction becomes only fast, when the ensuing radical cation is stabilized by electron donating substituents and the leaving group has a good nucleofugacity. In DNA, this kind of reaction leads to strand breakage from the *C*(4′) radical (Dizdaroglu et al. 1975). In poly(U), such reaction also occurs from the *C*(2′) radical [reaction (14); Hildenbrand and Schulte-Frohlinde 1989a,b]. Mechanistic details are discussed in Chapters 6.9 and 11.2.



9.4.5 $HO_2^{\bullet}/O_2^{\bullet-}$-Elimination Reactions and β-Fragmentation of Oxyl Radicals

In peroxyl-free-radical chemistry, $HO_2^{\bullet}/O_2^{\bullet-}$ elimination reactions play a major role (Chap. 8.4). In polymer free-radical chemistry, these reactions are of special interest, because they lead to a conversion of slowly diffusing polymer-derived radicals into the readily diffusing $HO_2^{\bullet}/O_2^{\bullet-}$ radicals. The $HO_2^{\bullet}/O_2^{\bullet-}$-elimination typically proceeds from an α-hydroxyalkylperoxyl radical [reaction (22)]. In poly(vinyl alcohol), for example, such an structural element is formed by H-abstraction and subsequent O_2 addition [reactions (18) and (19)]. The same structural element may also be formed during the bimolecular decay of peroxyl radicals which carry an H-atom in β-position [reactions (20) and (21)].

$$-HC(OH)\text{-}CR_2- + {}^{\bullet}OH \rightarrow -{}^{\bullet}C(OH)\text{-}CR_2- + H_2O \quad (18)$$

$$-{}^{\bullet}C(OH)\text{-}CR_2- + O_2 \rightarrow -{}^{\bullet}OOC(OH)\text{-}CR_2- \quad (19)$$

$$2\,-C(H,OO^{\bullet})\text{-}CR_2- \rightarrow 2\,-C(H,O^{\bullet})\text{-}CR_2- + O_2 \quad (20)$$

$$-C(H,O^{\bullet})\text{-}CR_2- \rightarrow -{}^{\bullet}C(OH)\text{-}CR_2- \quad (21)$$

$$-C(OH,OO^{\bullet})\text{-}CR_2- \rightarrow -C(O)\text{-}CR_2- + HO_2^{\bullet} \quad (22)$$

$$-C(OH,OO^{\bullet})\text{-}CR_2- + OH^- \rightarrow -C(O^-,OO^{\bullet})\text{-}CR_2- + H_2O \quad (23)$$

$$-C(O^-,OO^{\bullet})\text{-}CR_2- \rightarrow -C(O)\text{-}CR_2- + O_2^{\bullet-} \quad (24)$$

$$-C(H,O^{\bullet})\text{-}CR_2- \rightarrow -C(O)H + {}^{\bullet}CR_2- \quad (25)$$

The $HO_2^{\bullet}$ elimination at reaction (22) is often slow, and at a high concentration of peroxyl radicals this reaction may compete with the bimolecular decay of the peroxyl radicals (leading to chain scission; Ulanski et al. 1994). However in the presence of base, deprotonation speeds up the $O_2^{\bullet-}$ elimination [reactions (23)

and (24)]. Thus, under certain conditions, small changes in pH can have a dramatic effect on product yields in such systems.

It is noted that the 1,2-H shift [reaction (21)] always competes with the β-fragmentation reaction of oxyl-radicals [reaction (25)]. In the main chain of polymers, β-fragmentation causes a chain break. The ratio of the importance of reaction (21) vs. reaction (25) is determined by the stabilization of the radical that is released in the β-fragmentation process (25) (Gröllmann and Schnabel 1980; Schuchmann and von Sonntag 1982).

In DNA, the peroxyl radicals at *C*(3′)-*C*(5′) could, in principle, give rise to oxyl radicals that may lead to strand breakage through β-fragmentation. In contrast to the *C*(4′)-mechanism in the absence of O_2, details are, however, not yet established. For developing mechanistic concepts, we still fully rely on model systems such as those described above.

9.5 Oxidation and Reduction of Polymer Radicals

The reduction and oxidation of radicals are discussed in Chapter. 6.3–6.5. That in the case of radicals derived from charged polymers the special effect of repulsion can play a dramatic role was mentioned above, when the reduction of poly(U)-derived base radicals by thiols was discussed. Beyond the common oxidation and reduction of radicals by transition metal ions, an unexpected effect of very low concentrations of iron ions was observed in the case of poly(acrylic acid) (Ulanski et al. 1996c). Radical-induced chain scission yields were poorly reproducible, but when the glass ware had been washed with EDTA to eliminate traces of transition metal ions, notably iron, from its surface, results became reproducible. In fact, the addition of 1×10^{-6} mol dm^{-3} Fe^{2+} reduces in a pulse radiolysis experiment the amplitude of conductivity increase (a measure of the yield of chain scission; Chap. 13.3) more than tenfold and also causes a significant increase in the rate of the chain-breaking process. In further experiments, this dramatic effect of low iron concentrations was confirmed by measuring the chain scission yields by a different method. At present, the underlying reactions are not yet understood. These data are, however, of some potential relevance to DNA free-radical chemistry, since the presence of adventitious transition metal ions is difficult to avoid.

9.6 Products of the Termination of Polymer Radicals

Polymer radicals may recombine upon bimolecular termination. When this cross-linking takes place between two polymer molecules the molecular yield increases. When there are more than one radical on the polymer chain, cross-linking between these two sites will form only a loop (Fig. 9.1), that is, no increase in the molecular weight ensues despite the fact that cross-linking has occurred. In competition to recombination, radicals may disproportionate, when at least

one of the radicals carries a hydrogen in β-position. A disproportionation reaction does not lead to a change in molecular weight. The ratio of recombination to disproportionation depends considerably on the substituents next to the radical site (Chap. 6.11). Thus, a change from one kind of radical into another one (induced by an intramolecular radical transfer process, see above) must also have an influence on the recombination to disproportionation ratio and hence on the cross-linking yield. As a consequence, product yields (*inter*molecular cross-linking, loop formation, chain scission) strongly depend on the rate of radical generation and on the polymer concentration (at the same rate of radical generation but at lower polymer concentrations more radicals are formed per macromolecule and unit time than at a higher polymer concentration). Such a behavior is particular to polymers and usually not observed with low-molecular weight material.

Termination reactions of peroxyl radicals of polymers may eventually lead to chain scission (see above).

9.7 DNA

It has been discussed above that polymer radicals may have lifetimes different from those of their monomer units. These lifetimes have been shown to depend on various parameters such as charge and number of radicals per polymer chain. With respect to the lifetime of DNA radicals, not too many studies are available. In a very early pulse radiolysis study, it has been stated that very little UV/Vis absorption decrease of the DNA radicals over the time interval of 300-900 μs was detected (Scholes et al. 1969). Using Raleigh light-scattering for detection gave a somewhat complex picture (Lindenau et al. 1976). A relatively fast decrease in the signal with $t_{1/2} \approx 0.8$ ms was followed by a much slower one of $t_{1/2} \approx 8$ s. These changes were interpreted as being due to an detachment of DNA segments caused by DSBs and SSBs, respectively. If this interpretation is correct, these data would not lead to any information as to the lifetime of DNA radicals. In an experiment where a $\sim 3 \times 10^{-3}$ mol dm^{-3} calf thymus DNA solution was subjected to a short pulse of 350 Gy (equivalent to 2×10^{-4} mol dm^{-3} DNA radicals) and subsequently a tritiated nitroxyl radical (TAN) was added after some delay, a biphasic decay was observed, the slower part exhibiting a half-life of about 10 s (Brustad et al. 1971). It is evident that under these conditions quite a large number of radicals per DNA strand have been created (one per ten nucleotides, on average), and it is not unlikely that the rapid part has to be connected with the very fast early part of radical decay generally shown by polymers having a large number of radicals per polymer chain. In DNA, this fast decay will certainly slowed down by the repulsive forces of the phosphate groups.

For DNA in cells and in the absence of O_2, one has to take into account that the lifetime of the DNA radicals is not determined by their bimolecular decay but rather by their reaction with the cellular thiols, mainly GSH (Chap. 12.11). In the presence of O_2, the situation becomes more complex, and the lifetime of the DNA peroxyl radicals is as yet not ascertained. It is expected to be consider-

ably longer than the lifetime of DNA radicals in the absence of O_2. Already in small DNA fragments (Box et al. 1993, 1995; Tallman and Greenberg 2001) but also in DNA (Maccubbin et al. 2000; Bourdat et al. 2000), damage amplification reactions induced by one $^{\bullet}OH$ (tandem lesions) have been observed to take place quite effectively. This can only happen when either the intramolecular radical reaction is fast or the bimolecular termination reaction is slow. The latter certainly plays an important role here. In the case of DNA, O_2-uptake followed upon γ-irradiation of its aqueous solution is much higher than that of an equivalent mixture of its nucleotides (Isildar et al. 1982). This clearly shows that in the polymer additional reactions occur that are not given by its subunits.

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Nucleobases, Nucleosides and Nucleotides

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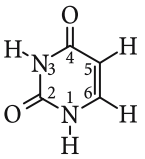
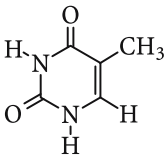
10.1 General Remarks

In DNA, there are four different nucleobases, the pyrimidines Thy and Cyt, and the purines Gua and Ade. In RNA, Ura replaces Thy[1].



Uracil (Ura)



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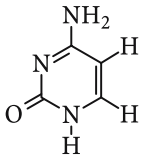
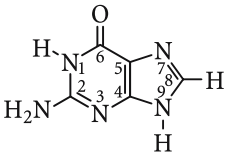
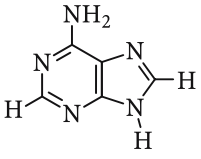


Cytosine (Cyt)



Guanine (Gua)



Adenine (Ade)

In their free-radical chemistry, these nucleobases have many properties in common. There are, however, also considerable differences which strongly affect the various reaction pathways. In nucleosides and nucleotides, free-radical attack mainly occurs at the base moiety. These reactions largely involve addition reactions. Only the sugar moiety and the methyl group in Thy can act as H-donors. The *C*(2′)-position is the least likely to be attacked because of the stronger BDEs of these hydrogens (Miaskiewicz and Osman 1994; Steenken et al. 2001), but this reaction can become of importance when favored by steric conditions.

10.1.1 pK_a Values

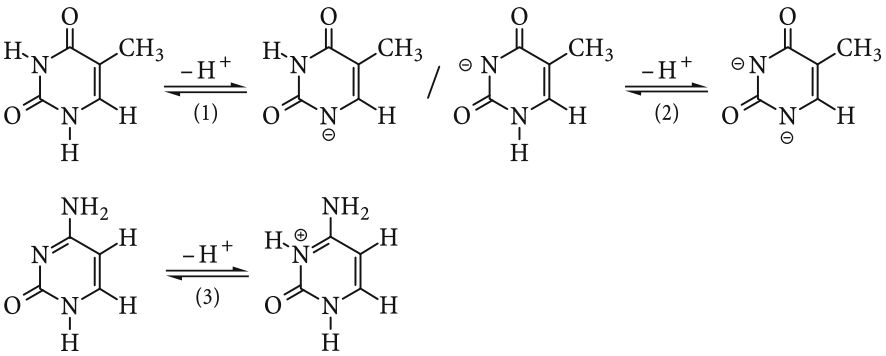
Ura, Thy and Gua deprotonate at pH < 10 [reaction (1)], but are not protonated at pH > 3. On the other hand, Cyt and Ade and their nucleosides already become protonated around pH 4 [e.g., reaction (3)], but, with the exception of Ade itself, they only deprotonate at very high pH (Table 10.1). The deprotonation of nucleosides at pH > 12 is often due to a deprotonation at the sugar moiety (Velikyan et

[1] In this chapter, the three-letter abbreviations (Fasman 1975) are used, and a few examples are given below. Nucleobases and its derivatives: adenine (**Ade**), guanine (**Gua**), cytosine (**Cyt**), thymine (**Thy**), uracil (**Ura**), 5-bromouracil (**5BrUra**), 1,3-dimethyluracil (**1,3Me$_2$Ura**), 5,6-dihydrouracil (**H$_2$Ura**), 5,6-dihydro,5,6-dihydroxythymine, thymine glycol, [**(OH)$_2$Thy**], 6-hydroxy-5,6-dihydrothymine (**6OHH$_2$Thy**). Nucleosides: adenosine (**Ado**), guanosine (**Guo**), Cytidine (**Cyd**), uridine (**Urd**), 2′-deoxyadenosine (**dAdo**), 2′-deoxyguanosine (**dGuo**), 2′-deoxycytidine (**dCyd**), thymidine (**Thd**), 2′-deoxy-5-bromouridine (**5BrdUrd**). Nucleotides: adenosine-3′-phosphate (**Ado-3′-P**), 2′-deoxyadenosine-5′-phosphate (**dAdo-5′-P**).

Table 10.1. pK_a values of nucleobases and nucleosides at the base moiety

Compound	Basic	Acidic
Ura	–	9.5/> 13
Urd	–	9.2
Thy	–	9.9/> 13
Thd	–	9.8
Cyt	4.6	12.2
dCyd	4.3	> 13
Ade	4.15	9.8
dAdo	3.8	–
Gua	< 0/3.2	9.6/12.4
dGuo	2.5	9.3

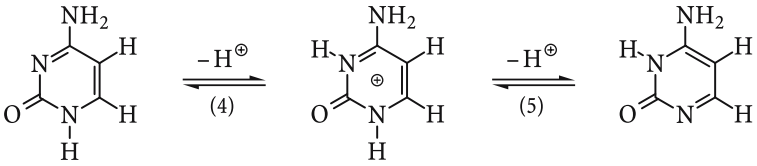
al. 2001). Double deprotonation [e.g., reaction (2)] only occurs at very high pH values an can be largely neglected.



The kinetics of the free-radical chemistry of the nucleobases has been studied by using mainly the pulse radiolysis technique (Chap. 13.3). For this reason, it is important to mention that short-lived transients are also observable in the case of Ura and Thy (Fielden et al. 1970; Greenstock et al. 1973b) as well as with Cyt (Greenstock et al. 1973b; Hissung and von Sonntag 1979; Schuchmann et al. 2005) even if all the water radicals are scavenged by other additives and thus are prevented from reacting with the nucleobase. The reason for this effect is the formation of H^+ and OH^- during the pulse (Chaps 2.2 and 13.3).

The pK_a values of Thy and Ura are close to 9.5. Thus, near neutrality they are deprotonated by OH^- formed in the pulse. Due to the low pK_a values, the reprotonation of the anions by water is sufficiently slow (~ 3×10^5 s^{-1}) to allow the anions to build-up to higher than steady-state concentrations. Of the two anions that are formed upon deprotonation [e.g., reaction (1)] only the one deprotonated at *N*(1) absorbs at longer wavelengths than the neutral molecules (Morita et al. 1981). This explains, why with the nucleosides which have the same pK_a values as the nucleobases no such intermediates are formed, although deprotonation by OH^- occurs as well.

With Cyt, the situation is quite different. The pK_a value of Cyt is 12.2. Hence, reprotonation of the Cyt anion by water is very fast (~10^8 s^{-1}), and the intermediate Cyt anion cannot reach a sufficiently high concentration to be observable under the conditions of such an experiment. On the other hand, Cyt is readily protonated, the pK_a value of the protonated Cyt being 4.6. The protonated Cyt absorbs at longer wavelengths than Cyt itself, and hence a short-lived intermediate is observed. This also applies to Cyd. However, while the subsequent deprotonation of the protonated Cyt by OH^- just reverts back to Cyd, this is not the case with Cyt (Schuchmann et al. 2005). There, a new absorption builds up due to the formation of its isomer, isocytosine, which has an absorption maximum at 296 nm (Dreyfus et al. 1976), i.e. at considerable longer wavelengths compared to Cyt (λ_{max} = 267 nm). Of the two isomers, Cyt is the thermodynamically favored species and isocytosine (richer in Gibb's free energy by 14 kJ mol^{-1}) eventually reverts to Cyt [reactions (4) and (5)].



Cytosine **Isocytosine**

10.1.2 Reduction Potentials

Gua has the lowest reduction potential among the four nucleobases (Table 10.2), and hence it is preferentially oxidized to its radical cation (for the calculation of ionization potentials of the DNA bases see Close 2004; Crespo-Hernández et al. 2004), and this property makes Gua and its derivatives to stick out of the other nucleobases with respect to its different free-radical chemistry. In contrast, Thy and Cyt are good electron acceptors, while the purines are only poor ones in comparison (for the calculation of electron affinities, see Richardson et al. 2004). This is of special importance in the effects caused by the absorption of ionizing radiation by DNA.

The reduction potential of Guo and Ado, determined pulseradiolytically (Steenken and Jovanovic 1997), corrects earlier much lower values (Jovanovic and Simic 1986, 1989), but is only slightly higher than the value of 1.47 V deter-

Table 10.2. Reduction potentials of nucleobases in nucleosides. (Steenken et al. 1992; Steenken and Jovanovic 1997; for further studies see text)

Compound	Reduction	Oxidation
Cyd	–1.15	
dCyd		$E_7 \approx 1.6$
Thd	–1.2	$E_7 \approx 1.7$
Ado		$E_7 = 1.56$
Guo		$E_7 = 1.29$

mined in acetonitrile by cyclic voltammetry (Seidel et al. 1996). Hydrogen bonding to Cyt reduces the reduction potential of Gua by 100 mV (Kawai et al. 2000; for further data including the reduction potentials of the nucleobases and some related compounds see Faraggi et al. 1996; for quantum mechanical calculations of electron affinities and ionization potentials of nucleobases see Faraggi and Klapper 1993; Russo et al. 2000).

The reduction potentials of all the nucleobase radical cations (and their deprotonated forms) are all higher than that of tryptophan ($E_7 = + 1.0$ V; Merényi et al. 1988; DeFelippis et al. 1989; Jovanovic et al. 1991), and in DNA repair of such intermediates by ET from surrounding proteins is thermodynamically feasible (for the reactions with other reducing agents see below). The DNA-binding bisbenzimidazole derivatives (such as *Hoechst 33258*) are expected to have even lower reduction potentials, and these compounds undergo efficient ET not only to one-electron oxidized purines on the model level (Adhikary et al. 1997b, 2000; Martin and Anderson 1998) but also when bound to DNA (Adhikary et al. 1997a).

Among the nucleobases, Thy and Cyt are most readily reduced (Table 10.2). Electron attachment energies have been determined in the gas phase (Aflatooni et al. 1998), and they confirm this view. Theoretical vertical attachment energy calculations rank the nucleobases in the order Ura < Thy < Cyt < Ade < Gua (Sevilla et al. 1995).

10.2 Radical Cations and their Conjugate Bases, the Heteroatom-Centered Radicals

10.2.1 Formation of Radical Cations

In the direct effect of ionizing radiation on DNA, radical cations are the primary products (Chap. 12). For this reason, their reactions are of considerable interest. Obviously, photoionization (e.g., at 193 nm) and laser multi-photon excitation leads to such species (e.g., Candeias and Steenken 1992b; Malone et al. 1995; Chap. 2.2). Base radical cation electron pairs have been proposed to be the first observable intermediates with a lifetime of 10 ps for Ade and four times longer for the other nucleobases (Reuther et al. 2000). Radical cations are also assumed to be intermediates in the reactions of photosensitization reactions with quinones, benzophenone, phthalocyanine and riboflavin (Cadet et al. 1983a; Decarroz et al. 1987; Krishna et al. 1987; Ravanat et al. 1991, 1992; Buchko et al. 1993; Douki and Cadet 1999; Ma et al. 2000). Nucleobase radical cations may be produced by electrochemical oxidation (Nishimoto et al. 1992; Hatta et al. 2001) or with strongly oxidizing radicals (for a compilation of their reduction potentials see Chap. 5.3). Rate constants are compiled in Table 10.3.

One-electron oxidation of dGuo to its radical cation, $G^{\bullet+}$, is achieved by strong oxidants such as Tl^{2+}, $SO_4^{\bullet-}$ and $Br_2^{\bullet-}$ (Table 10.3; for its EPR spectrum generated by $SO_4^{\bullet-}$ see Bachler and Hildenbrand 1992) as well as the heteroatom-centered radicals derived from the other nucleobases (e.g., Shi et al. 2000a). Weaker oxidants such as $N_3^{\bullet}$ and the dimeric tetramethylthiourea radical cation are capable of oxidizing dGuo only at high pH, i.e. in its anionic form thereby producing the guanyl radical, $G^{\bullet}$ (Schuchmann et al. 2000). Depending on the nature of the oxidant, oxidation may take place by direct ET as well as by addition-elimination. Kinetic deuterium isotope effects of 1.5-2 are observed in the (reversible) oxidation of Gua by 2-aminopurine radicals, and it has been concluded that this redox equilibrium can be considered in terms of a proton-coupled ET (Shafirovich et al. 2000). Such a proton-coupled ET step leads to a lowering of the overall free energy of reaction thus favoring ET (Rehm and Weller 1970; Atherton and Harriman 1993; for theoretical calculations see Cho and Shin 2000 and references cited therein).

Short-lived adducts may be formed as intermediates in the reactions of the oxidizing inorganic radicals with the nucleobases, and it is therefore not always fully excluded that processes observed at very short times and attributed to the reactions of radical cations are in fact due to such intermediates. It may be mentioned that, for example, a long-lived $SO_4^{\bullet-}$-adduct is observed in the reaction of $SO_4^{\bullet-}$ with maleic acid (Norman et al. 1970). It has been suggested that $SO_4^{\bullet-}$ in its reactions with the pyrimidines forms only an adduct and does not give rise to radical cations (Lomoth et al. 1999). The observation of heteroatom-centered radicals by EPR from the nucleobases Ura, Thy and Cyt (Catterall et al. 1992) as well as dCyd (Hildenbrand et al. 1989) (see below) has been taken as evidence that in the reaction of $SO_4^{\bullet-}$ with pyrimidines radical cations are likely, albeit

Table 10.3. Compilation of rate constants (unit: $dm^3\ mol^{-1}\ s^{-1}$) of oxidizing radicals with nucleobases and related compounds

Substrate	Radical	Rate constant	Reference
Ade	$SO_4^{\bullet-}$	4.6×10^9	Vieira and Steenken (1987a)
9MeAde	$SO_4^{\bullet-}$	4.1×10^9	Vieira and Steenken (1987a)
dAdo	$SO_4^{\bullet-}$ $Br_2^{\bullet-}$	3.2×10^9 4×10^5	Steenken (1989) von Sonntag (1994)
dAdo/H^+ (pH 2.4)	$SO_4^{\bullet-}$	4.4×10^9	Steenken (1989)
Ado	$SO_4^{\bullet-}$ Tl(II)	2.7×10^9 6.3×10^7	Vieira and Steenken (1987a) Al-Sheikhly (1994)
N^6,$N^6$$Me_2$Ado	$SO_4^{\bullet-}$	3.9×10^9	Vieira and Steenken (1987a)
dCyd	$SO_4^{\bullet-}$	2.5×10^8 1.6×10^9	O'Neil and Davies (1987) Aravindakumar et al. (2003)
Cyd	$SO_4^{\bullet-}$	3×10^9	Aravindakumar et al. (2003)
Cyt anion	$SO_4^{\bullet-}$	7.5×10^8	Hazra and Steenken (1983)
Cyt/H^+ (pH 2)	$Cl_2^{\bullet-}$	1.0×10^7	Patterson et al. (1972)
dCyd/H^+ (pH 2)	$Cl_2^{\bullet-}$	4×10^6	Patterson et al. (1972)
Gua anion	$^{\bullet}N_3$	4×10^9	Faraggi and Klapper (1994)
dGuo	$SO_4^{\bullet-}$ $SeO_3^{\bullet-}$	2.3×10^9 4.1×10^9 1.2×10^9	O'Neill and Davies (1987) Steenken (1989) Martin and Anderson (1998)
dGuo anion	$^{\bullet}N_3$ $(Me_4Thiourea)_2^{\bullet+}$	4×10^9 1.2×10^8	Faraggi and Klapper (1994) Schuchmann et al. (2000)
dGMP	$Br_2^{\bullet-}$	4×10^7	Willson et al. (1974)
Guo	Tl(II)	1.26×10^9	Al-Sheikhly (1994)
Thy	$SO_4^{\bullet-}$ $Cl_2^{\bullet-}$ $HPO_4^{\bullet-}$	3.1×10^9 7.0×10^7 9.6×10^7	Fujita et al. (1988) Patterson et al. (1972) Nakashima and Hayon (1979)
Thd	$SO_4^{\bullet-}$	2.1×10^9	Deeble et al. (1990)
3MeThd	$SO_4^{\bullet-}$	3.5×10^9	Deeble et al. (1990)
TMP	$SO_4^{\bullet-}$	1×10^9	Deeble et al. (1990)
1MeThy	$SO_4^{\bullet-}$	5×10^9	Deeble et al. (1990)
1,3Me_2Thy	$SO_4^{\bullet-}$	4.6×10^9	Deeble et al. (1990)
Ura	$Cl_2^{\bullet-}$ $HPO_4^{\bullet-}$ $H_2PO_4^{\bullet}$	3.5×10^7 9.7×10^7 6×10^8	Patterson et al. (1972) Nakashima and Hayon (1979) Nakashima and Hayon (1979)
1,3Me_2Ura	$SO_4^{\bullet-}$	5.5×10^9	Schuchmann et al. (1987)
1,3,6Me_3Ura	$SO_4^{\bullet-}$	3.5×10^9	Deeble et al. (1990)

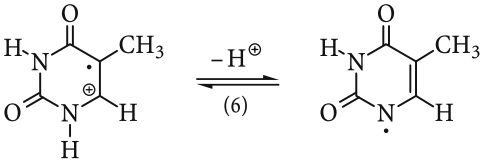
possibly very short-lived, intermediates. Yet, concerted release of sulfate ion and a proton would lead to the same heteroatom-centered radicals without free radical cations as intermediates (Aravindakumar et al. 2003). Once the heteroatom-centered radicals are formed, their protonation will lead to the formation of radical cations. Thus, such intermediates may play a role in the subsequent chemistries even if not formed in the primary step. This may, for example, be the reason for the formation of the allylic radical in the reaction of $SO_4^{\bullet -}$ observed with Thy (Deeble et al. 1990).

$Br_2^{\bullet -}$ can also be used to oxidize good electron donors, but at least with the pyrimidines its rate of reaction is too slow to be of any importance. Instead, degradation may occur by Br_2, the product of the disproportionation of $Br_2^{\bullet -}$, as has been shown for Thd (Cadet et al. 1983b).

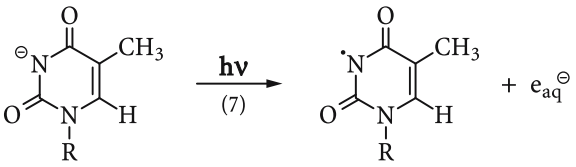
10.2.2 pK_a Values of Radical Cations and Heteroatom-Centered Radicals

Usually, radical cations have much lower pK_a values than their parent compounds. A typical examples is phenol, whose pK_a value is at 10 while that of its radical cation is at -2 (Dixon and Murphy 1976). Thus in this case, ionization causes an increase in acidity by 12 orders of magnitude. It is hence expected that also the nucleobase radical cations should be much stronger acids than their parents. This has indeed been found in all systems where equilibrium conditions are established, and the consequences for DNA base pairs have been discussed (Steenken 1992).

Pyrimidines. Photoexcited anthraquinone-2,6-disulfonate undergoes ET with Thy and its methyl derivatives as indicated by Fourier transform EPR (Geimer et al. 1997). These pyrimidine radical cations deprotonate at *N*(1) thereby giving rise to the corresponding *N*-centered radicals [reaction (6)].

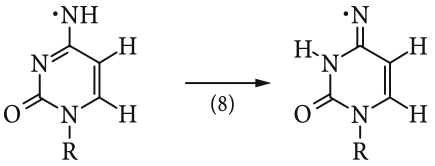


This view is been confirmed by an electrochemical product study (Hatta et al. 2001) that is discussed below. The pK_a value of the Thy radical cation has been determined at 3.2 (Geimer and Beckert 1998). When the position at *N*(1) is substituted by a methyl group and deprotonation of the radical cation can no longer occur at this position, deprotonation occurs at *N*(3) (Geimer and Beckert 1999; for spin density calculations using density functional theory (DFT) see Naumov et al. 2000). This *N*(3) type radical is also produced upon biphotonic photoionization of *N*(1)-substituted Thy anions [reaction (7)] in basic 8 molar $NaClO_4$–D_2O glasses which allowed to measure their EPR spectra under such conditions (Sevilla 1976).

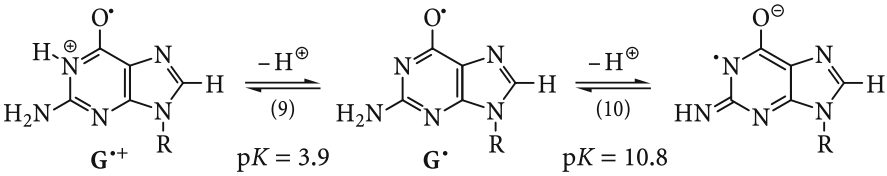


From a pulse radiolysis study on the $SO_4^{\bullet -}$-induced reactions of Thd (Deeble et al. 1990), it has been concluded that the pK_a of the Thd radical cation (deprotonation at *N*(3)) should be near 3.5, i.e. close to that at *N*(1) in Thy. It is noted that also in the parent, Thy, the pK_a values at *N*(1) and at *N*(3) are quite close. A Fourier-transform EPR study using photoexcited anthraquinone-2,5-disulfonic acid to oxidize Cyt and 1MeCyt shows that the radical cation of the former deprotonates rapidly at *N*(1) while that of the latter deprotonates at the exocylic amino group (Geimer et al. 2000). The EPR evidence for the formation of heteroatom-centered radicals by $SO_4^{\bullet -}$ in its reactions with some other pyrimidines (Bansal and Fessenden 1978; Hildenbrand et al. 1989; Catterall et al. 1992) is in agreement with a marked acidity of such radical cations. It is re-emphasized that this conclusion does not require that radical cations are formed in the primary step.

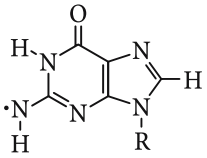
The primary exocyclic *N*-centered dCyd radical is only observed at very short times and is converted into another radical which has been attributed (Naumov et al. 2001) to its tautomer [reaction (8)]. This tautomerization has been calculated to be exothermic by 10.5 kJ mol^{-1}.



Purines. The Gua radical cation, $G^{\bullet +}$, is an acid (pK_a = 3.9; Willson et al. 1974; Asmus et al. 1978) and readily deprotonates at a heteroatom. In water, the deprotonation product identified by pulse radiolysis is the *N*(1) radical [denoted as $G^{\bullet}$; equilibrium (9); Candeias and Steenken 1989].



The radical that is formed upon deprotonation at *N*(2), *N*(2)$G^{\bullet}$, is very close in energy.

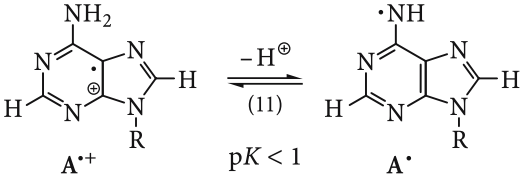


N(2)G•

In fact, calculations show that in the gas phase *N*(2)G• is more stable than G• (Mundy et al. 2002; Naumov et al., unpubl. results; in Wetmore et al. 1998a apparently an energy-richer rotamer (local minimum) has been calculated). The cross-over in stability from *N*(2)G• to G• would occur in a solvent having a DK like MeOH, and in γ-irradiated crystals of dGMP and cyclo-GMP *N*(2)G• (and not G•) is the stable species formed upon deprotonation of $G^{\bullet+}$ (Hole et al. 1987, 1989, 1992a,b; Close et al. 1985; Nelson et al. 1988). The reason for a change-over in stability as a function of solvent (environment) polarity is the marked difference of the dipole moments of G• and *N*(2)G• which has been calculated at 5.5 Debye (in water; Naumov et al., unpubl. results). This may now also explain the results obtained when GMP is oxidized in aqueous solution by photoexcited tris(1,4,5,8-tetraazaphenanthrene)ruthenium(II) (Jaquet et al. 1995). After deprotonation of $G^{\bullet+}$ in the solvent cage, the resulting *N*(2)G• adds to the ligand of the Ru-complex forming a well-characterized product. Since all these reactions are believed not to occur in the bulk but in the solvent cage where the partners, GMP and the Ru-complex with its aromatic ligands provide an environment whose effective DK will be considerably below that of water, *N*(2)G• is the stable species under such conditions.

At high pH, G• deprotonates further [equilibrium (10), $pK_a(G^{\bullet}) = 10.8$; Candeias and Steenken 1989]. $G^{\bullet+}$ ($pK_a = 3.9$) is a weaker acid than the protonated parent ($pK_a = 2.4$) but a stronger acid than its corresponding neutral parent ($pK_a = 9.4$). Based on the redox potential and the pK_a value, the *N*(1)–H binding energy in dGuo has been calculated at 380 kJ mol^{-1} (Steenken et al. 2001).

Ade has a considerably higher reduction potential than Gua (Table 10.2), and for this reason it is not as readily oxidized, even by strongly oxidizing radicals, $SO_4^{\bullet-}$ excepted. The pK_a of the dAdo radical cation, $A^{\bullet+}$, lies at less than 1 [equilibrium (11); Steenken 1989; for gas phase data see Hwang et al. 1999].

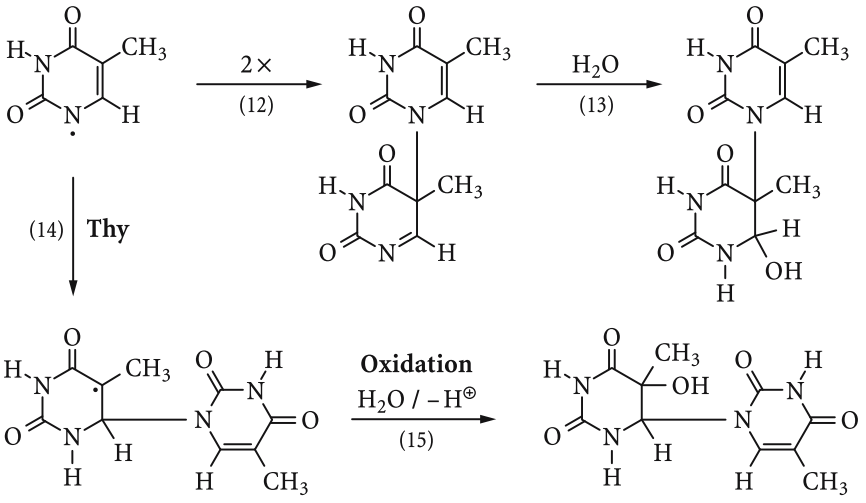


$A^{\bullet+}$ $A^{\bullet}$

10.2.3 Reactions of $SO_4^{\bullet -}$/$PO_4^{\bullet 2-}$-Adduct Radicals, Radical Cations and Heteroatom-Centered Radicals

Radical cations are strongly oxidizing intermediates, but also after deprotonation at a heteroatom (in the present systems at nitrogen) some of this oxidizing property remains. Thus a common feature of these intermediates is that they are readily reduced by good electron donors. Since the heteroatom-centered radicals and the radical cations are always in equilibrium, it is, at least in principle, possible that such intermediates react with water at another site (canonical mesomeric form), that is at carbon. This reaction leads to •OH-adduct radicals. Although deprotonation at a heteroatom is usually faster (but also reversible) than deprotonation at carbon, the latter reaction is typically "irreversible". This also holds for a deprotonation at methyl (in Thy).

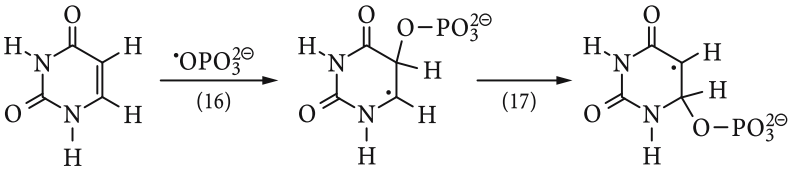
Pyrimidines. Reaction of Thy with photoexited menadione or its electrochemical oxidation yields mainly to the *N*(1)-*C*(5)-linked dimer (Hatta et al. 2001). This can be accounted for if the precursor radical cation deprotonates at *N*(1) (see above). For this *N*(1)-centered radical a second mesomeric form with the spin at *C*(5) can be written. Head-to-tail recombination leads to the isopyrimidine-type dimer [reaction (12)]. Isopyrimdines are unstable (see below) and add rapidly water [reaction (13)]. This dimer is also formed in the reaction with $SO_4^{\bullet -}$, albeit with a lower yield.



Upon electrochemical oxidation, dimers resulting from a recombination at *C*(5) are formed as well. Whether this route is mediated by the electrode surface or due to a recombination of a radical cation with an *N*-centered radical (note that there is a high radical density at the electrode surface and that the radical cation (pK_a = 3.2) has a lifetime of ~0.5 µs), must remain speculation. To a smaller extent, a further dimer is also observed. It may arise by an addition/oxidation

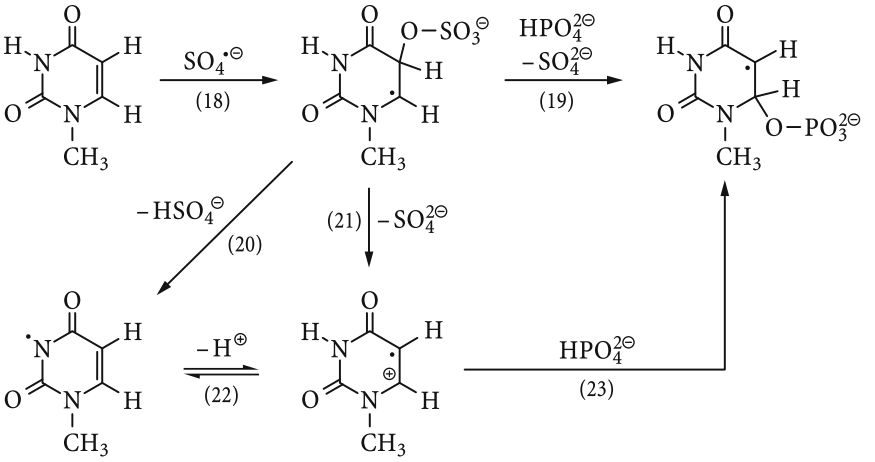
mechanism [reactions (14) and (15); a similar sequence has also been envisaged for the formation of the major dimer; Hatta et al. 2001]. An alternative route to this minor dimer would be a reaction of the radical cation with water (in competition with its deprotonation) yielding the *C*(5)OH-*C*(6)-yl radical and its recombination with the major radical. Since the addition of water to the radical cation would only be a minor route, it may easily have escaped detection by EPR. The formation of dimers have also been observed by electrochemical oxidation of 5FUra and 5ClUra, but 5BrUra and 5IUra did not afford any dimers (Hatta et al. 2001).

The electrophilic $SO_4^{\bullet -}$ preferentially adds to an electron-rich position of the substrate, *C*(5) in the case of pyrimidines. This must also hold for the phosphate radical [reaction (16)]. Here, however, the radical observed by EPR is the *C*(6)-adduct radical, and it has been concluded that a rapid 1,2-shift leads to this thermodynamically more stable radical [reaction (17); Behrens et al. 1988]. At pH > 6.5, the transformation reaction (17) is sufficiently slow to be intercepted by a spin trap, and the adduct to the primary radical formed in reaction (16) is indeed observed (Hildenbrand 1995).



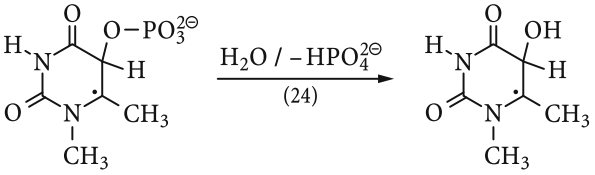
For a mechanistic interpretation of the EPR data, one must keep the possibility in mind that the reducing *C*(5)-adduct radical may have been oxidized by peroxodiphosphate in the free-radical chain reaction (see below) and that only the oxidizing *C*(6)-adduct radical remains in this sequence of reactions, although formed in small amounts, and is eventually detected by EPR as the only radical.

The phosphate-adduct radical is also formed, when the reaction is initiated by $SO_4^{\bullet -}$ [reaction (18)] in the presence of phosphate ions (Behrens et al. 1988). This may either be due to an S_N2 substitution reaction [reaction (19)] or a reaction of the phosphate ion with the radical cation [reaction (17)] formed either by an elimination of SO_4^{2-} plus H^+ [reaction (20)] and subsequent protonation of the *N*(3)-centered radical [equilibrium (22)] or by SO_4^{2-} elimination [reaction (21)], as envisaged originally. The reaction of the radical cation with phosphate would then give rise to the observed radical [reaction (23)].



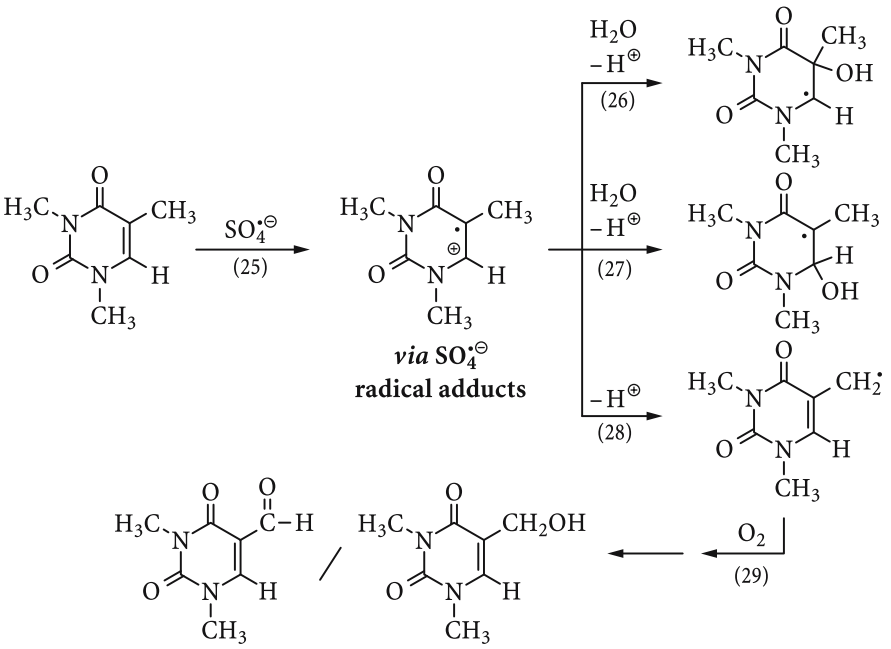
From the competition of phosphate and water for the intermediate(s) formed by $SO_4^{\bullet -}$, it has been concluded that the rate constant of reaction with phosphate must be $\geq 4 \times 10^8$ dm^3 mol^{-1} s^{-1}. The transformations of intermediates that play a role in this system by OH^- and by phosphate were followed by EPR techniques (Hildenbrand et al. 1989; Geimer and Beckert 1999).

When the electron-donating methyl substituent is introduced at *C*(6), the *C*(6)-phosphate-adduct radical is no longer stable on the EPR time scale, and



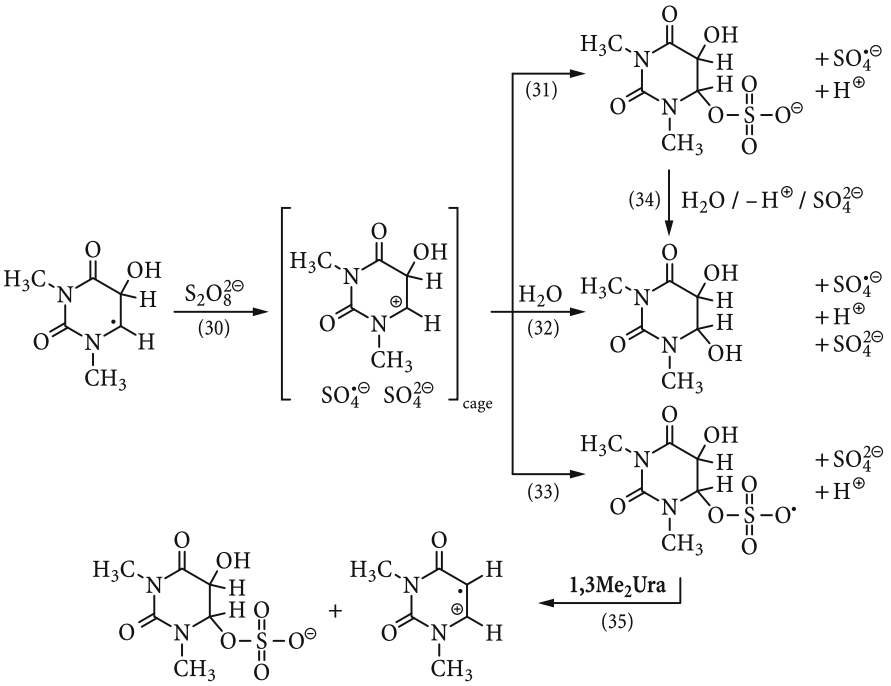
only its hydrolysis product is observed [reaction (24); Behrens et al. 1988]. The reason for the greater stability of the phosphate radical-adduct compared the sulfate radical-adduct is due to effects discussed in Chapter. 6.9.

It has been mentioned above that the pyrimidine radical cations are reasonably strong acids and rapidly deprotonate at a heteroatom. As all protonation/deprotonation reactions at heteroatoms are reversible [e.g., equilibrium (22)], the radical cations are regenerated upon reprotonation. Deprotonation at carbon or reaction with water yield the final free-radical products. For the 1,3Me$_2$Thy system, where the deprotonation/reprotonation equilibria such as reaction (22) fall away, reactions (25)-(28) have been postulated to account for the fact that in the presence of O_2 1,3Me$_2$5HOMeUra and 1,3Me$_2$5(CHO)Ura [reaction (29)] are formed in a combined yield of 80% of primary $SO_4^{\bullet -}$ radicals (Rashid et al. 1991). The formation of these products has been taken as evidence that a free radical cation must be an intermediate. It is, however, also possible that the allylic radical is formed in a concerted reaction HSO_4^- elimination. For such a process, a six-membered transition state can be written.



The allylic Thy radical is observed by EPR in Thd (Catterall et al. 1992) and TMP (Hildenbrand 1990). The identification of the *C*(6)-OH-5-yl radical by EPR supports the view (Deeble et al. 1990) that reaction with water competes with a deprotonation at methyl. Due to the ready oxidation of the (reducing) *C*(5)-OH-6-yl radicals by peroxodisulfate, this type of radical is only observed at low peroxodisulfate concentrations in these systems, i.e. the (oxidizing) *C*(6)-OH-5-yl radicals may be correspondingly enriched (Schulte-Frohlinde and Hildenbrand 1989) (note that the nucleobases themselves are not oxidized at a reasonable rate unless deprotonated; Moschel and Behrman 1974). These reducing *C*(5)-OH-6-yl radicals are capable of reacting with peroxodisulfate and thus induce chain reactions which in the case of 1,3Me$_2$Ura shows some very interesting properties (Schuchmann et al. 1987). It is nearly independent of the peroxodisulfate concentration, but shows a marked dependence on the 1,3Me$_2$Ura concentration. From this, it immediately follows that the mechanism is *not* characterized by the reaction of the reducing *C*(5)-OH adduct radical with peroxodisulfate as the rate determining step [reaction (30), $k = 2.1 \times 10^5$ dm^3 mol^{-1} s^{-1}], yielding *exclusively* SO_4^{2-}, the glycol [via the carbocation and water, reaction (32)]. This would be the trivial case of an $SO_4^{\bullet-}$-induced chain reaction (cf. Schuchmann and von Sonntag 1988; Ulanski and von Sonntag 1999). One rather has to consider that in this reaction the 1,3Me$_2$Ura carbocation, $SO_4^{\bullet-}$ and SO_4^{2-} are formed within the cage [reaction (30)]. There, the carbocation may recombine with either of the two anions or react with water [reactions (31)–(33)]. When the carbocation reacts with $SO_4^{\bullet-}$ [reaction (33)], a new oxidizing species is formed which, however, is not as reactive as $SO_4^{\bullet-}$. It propagates the chain with only a slow rate

[reaction (35), $k = 1.2 \times 10^4$ dm^3 mol^{-1} s^{-1}], and thus the chain length becomes dependent on the 1,3Me$_2$Ura concentration.



The same type of chain reaction is observed in the case of 1,3,6Me$_3$Ura with one minor difference: while with 1,3Me$_2$Ura the only chain product is the glycol, there are two chain products in 1,3,6Me$_3$Ura, its glycol and 1,3,6-trimethylisobarbituric acid, formed in a ratio of 2:1 (Rashid et al. 1991). The latter is believed to arise from the sulfate by an elimination of sulfuric acid. A deprotonation at methyl does not take place. This is quite in contrast to the situation in 1,3Me$_2$Thy and other Thy systems discussed above.

A chain reaction is also apparent in the reaction of $SO_4^{\bullet -}$ with Thy, since the quantum yield of Thy destruction exceeds $\Phi = 2.0$ in the photolysis of peroxodisulfate in the presence of Thy (Sudha Sawargara and Adinarayana 2003).

In pulse radiolysis studies of Urd and its derivatives (but not with dUrd), spectral changes are observed after the completion of the $SO_4^{\bullet -}$ reaction [$k = 3 \times 10^5$ s^{-1}; Bothe et al. 1990] that are not typical for $SO_4^{\bullet -}$ reactions with pyrimidines. On the basis of EPR experiments (Hildenbrand 1990; Catterall et al. 1992), these observations can be interpreted by an (overall) intramolecular H-transfer giving rise to a radical at the sugar moiety. This requires that considerable amounts of Ura are released which is indeed observed (Fujita et al. 1988; Aravindakumar et al. 2003; Table 10.4). Chain reactions occur as with the other pyrimidine/peroxodisulfate systems. This increases the Ura yield beyond that expected for a non-chain process, but when corrections are made for this by carrying out experiments at the very high dose rates of electron-beam irradiation, a

Table 10.4. *G* (base release) (unit: 10^{-7} mol J^{-1}) from some pyrimidine nucleosides and 2′-deoxynucleosides induced by the $SO_4^{\bullet-}$ radical [$G(SO_4^{\bullet-}) = 3.3 \times 10^{-7}$ mol J^{-1}) at different dose rates: pulsed electron-beam irradiation (~6 Gy per 2 µs pulse, high dose rate) and γ-irradiation (0.013 Gy s^{-1}, low dose rate; Aravindakumar et al. 2003)

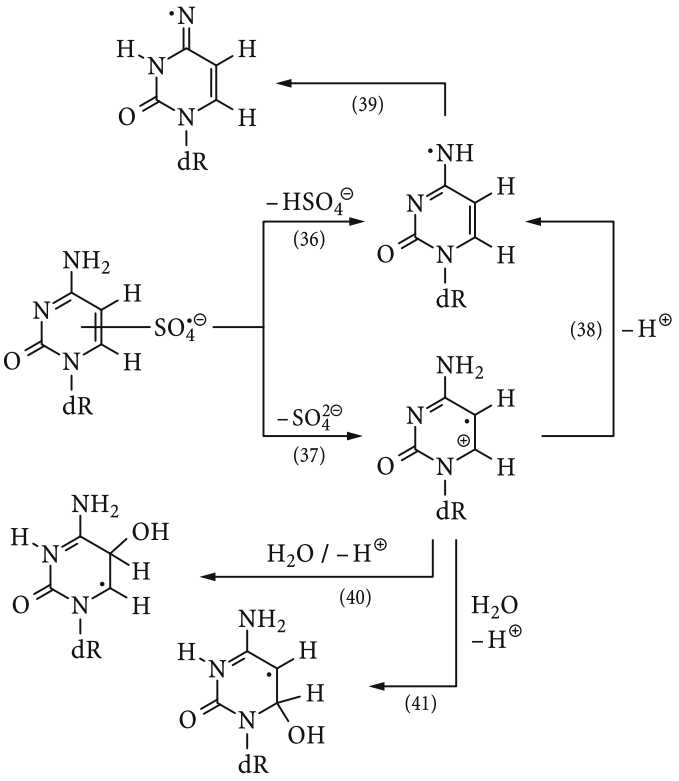
Nucleoside/2′-deoxynucleoside	Electron beam	γ-Radiolysis
Cyd	2.8	7.5
Urd	0.72	4.5
5MeUrd	n.d.	<0.05
dCyd	0.14	1.64
dUrd	0.06	0.6
Thd	0.2	1.0[a]

n.d., Not determined
[a] 0.7×10^{-7} mol J^{-1} at 1.3 Gy s^{-1}, 0.85×10^{-7} mol J^{-1} at 0.1 Gy s^{-1}

substantial part (22% of primary $SO_4^{\bullet-}$) of Ura release must be due to this radical transfer from the base to the sugar moiety.

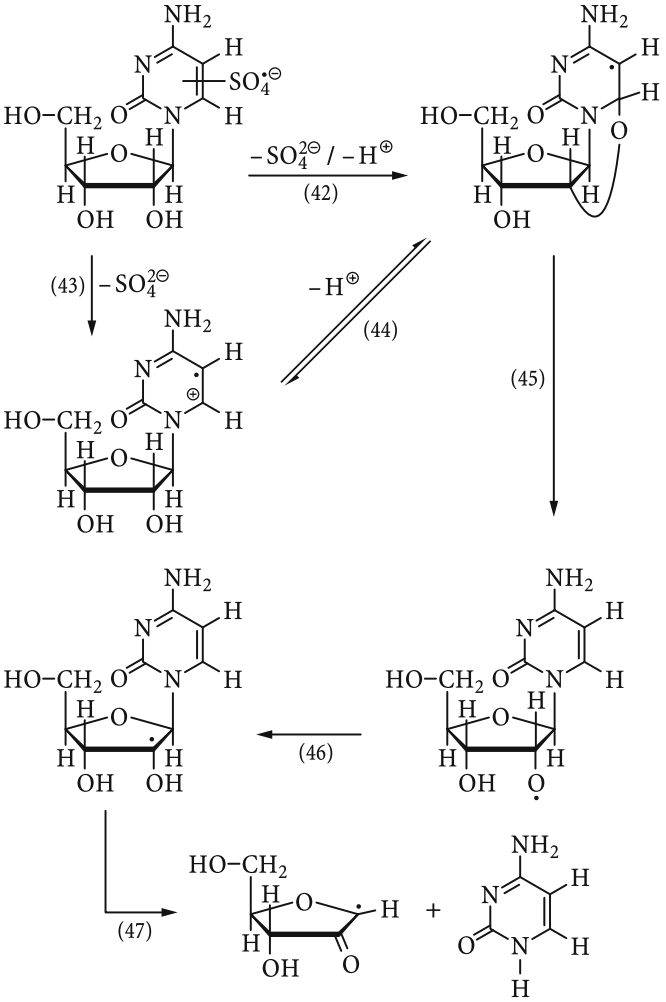
Mechanistic details will be discussed below, since the Urd seems to be analogous to the Cyd system, and the latter has been investigated in more detail (Schulte-Frohlinde and Hildenbrand 1989; Hildenbrand 1990; Catterall et al. 1992; Naumov et al. 2001; Aravindakumar et al. 2003). Interestingly, 5MeUrd does not undergo base release (Table 10.4), indicating that in this system the pathways dominating the reactions of Urd and Cyd are not followed. Whether the oxidation at methyl is the competing reaction is not yet known. A detailed mechanistic study would be required to shed light into this interesting phenomenon.

dCyd and Cyd differ dramatically in their reactions with $SO_4^{\bullet-}$. dCyd gives rise to a base-centered radical [reactions (36)–(41); Hildenbrand et al. 1989] which is formed in the tautomerization reaction (39) (Naumov et al. 2001).



In the original mechanistic concept, a free radical cation, reaction (37), has been postulated (Catterall et al. 1992). Based on circumstantial evidence, the concomitant release of HSO_4^- is now favored [reaction (36); Aravindakumar et al. 2003].

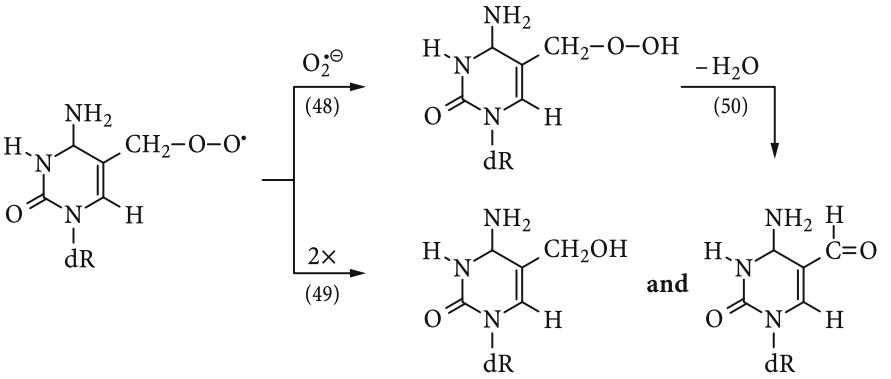
Cyd, in contrast, gives rise to a sugar-centered radical. The mechanism proposed by (Catterall et al. 1992) has been modified by Aravindakumar et al. (2003) [reactions (42)–(47)] insofar as route (42) is now favored over (43) followed by (44).



The S_N2 reaction (42) involving the *C*(2′)OH group competes successfully with the analogous reactions of water [cf. reactions (40) and (41)]. The ensuing radical rapidly decays by β-fragmentation [reaction (45), $k = 6 \times 10^4\ s^{-1}$; Schulte-Frohlinde and Hildenbrand 1989; Aravindakumar et al. 2003]. The 1,2-H-shift of the oxyl radical [reaction (46)] will be fast (Chap. 7.2). The radical that is detected by EPR requires that reaction (47) is also relatively fast. The Cyt yield is 85% of the primary $SO_4^{\bullet-}$ yield (cf. electron beam experiments in Table 10.4). Phosphate interferes with this sequence of reactions, and the same type of base radical as observed with dCyd now predominates (Niehaus and Hildenbrand 2000). The interference occurs twice, once by deprotonating the primary $SO_4^{\bullet-}$-adduct radical [enhancing the rate of reaction (42)] and also by protonating the cyclized radical [reaction (45)], i.e. it also shortens the lifetime of the latter (Aravindakumar et al. 2003).

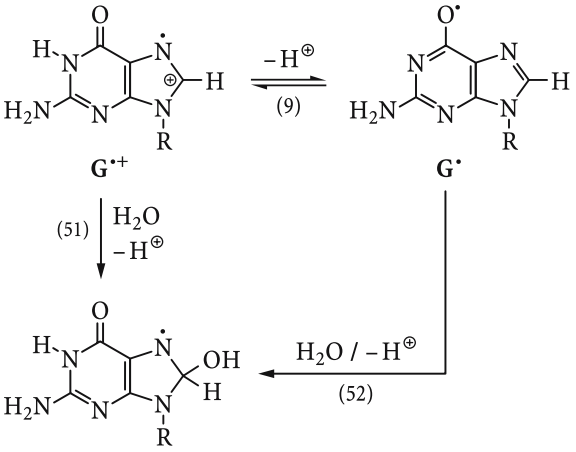
The menadione-sensitized oxidation of dCyd leads to the formation of the four *cis* and *trans* diasteroisomers of $(OH)_2$dUrd and to ring-opened products, and it has been concluded that the major reaction of the dCyd radical cation is its reaction with water yielding the •OH-adduct radical (Decarroz et al. 1987). However, *N*-centered radicals could well be their precursors. The formation of Cyt and 2-dRL has also been noticed.

The corresponding reaction with 5MedCyd leads mainly to an oxidation of the methyl group via a deprotonation of the radical cation at methyl (Bienvenu et al. 1996). Evidence for such a reaction has been obtained by EPR at 77K using biphotonic excitation to generate the radical cation (Malone et al. 1995). Mechanistically, this is analogous to the situation of Thy and its derivatives [reaction (28)]. In the presence of O_2, the 5MedCyd-derived allylic radical gives rise to the corresponding peroxyl radical. Upon its bimolecular decay, 5-hydroxymethyl-dCyd and 5-formyl-dCyd are formed [reaction (49)]. Moreover, $HO_2^•/O_2^{•-}$ (resulting from the reaction of the menadione radical anion with O_2) reduce the peroxyl radical to the hydroperoxide [reaction (48)] which decays into 5-formyl-dCyd with a half-life of 9.2 h by water elimination [reaction (50); Bienvenu et al. 1996].



For a study on the reaction of pyrimidine bases with the phosphate radical in the presence of peroxodiphosphate see Kumar et al. (2000); Kumar and Adinarayana (2001).

Purines. According to DFT calculations, the reaction of $G^{•+}$ with water [reaction (51)] is exothermic by 315 kJ mol^{-1}, while a corresponding reaction of the G• formed upon deprotonation of $G^{•+}$ is endothermic by 123 kJ mol^{-1}, and thus reaction (52) is no longer possible (Reynisson and Steenken 2002). Similarly, the reaction of $A^{•+}$ with water is exothermic by 325 kJ mol^{-1}, while A• formed upon deprotonation can no longer undergo such an reaction due to a calculated endothermicity of 183 kJ mol^{-1}.



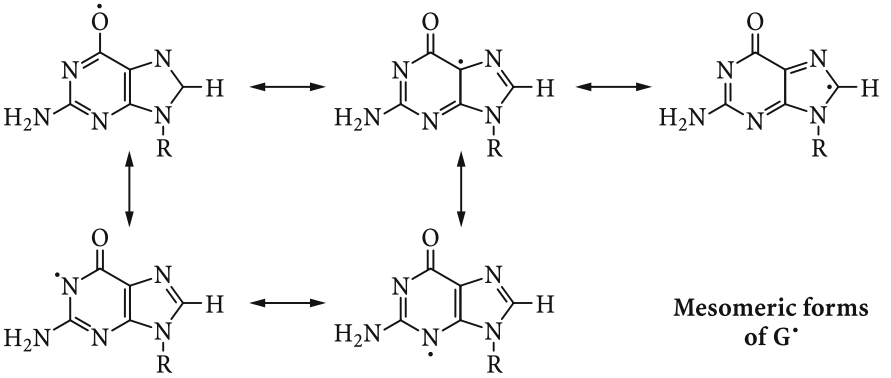
In the nucleoside and in ssDNA, $G^{\bullet+}$ ($pK_a = 3.9$) is expected to loose the proton rapidly to the water phase, but in dsDNA pairing with Cyt will prolong its lifetime and reaction (51) may proceed with a higher efficiency. The resulting *C*(8)-•OH-adduct is the precursor of the 8-oxo-G and other well-documented lesions (see below). The same type of reaction is expected to occur with $A^{\bullet+}$ (see below), but in dsDNA Ade binding to Thy will not prevent its deprotonation at N^6. This may be one of the reasons why the 8-oxo-G lesion is of a higher importance than the 8-oxo-A lesion (Chap. 12.9).

$G^{\bullet}$ is readily reduced by electron donors such as TMPD, ascorbate, ABTS (O'Neill and Chapman 1985), phenolic compounds (Jiang et al. 1999a,b; Shi et al. 1999b) or bisbenzimidazole derivatives (the *Hoechst* group of fluorescent dyes; Adhikary et al. 1997b; Martin and Anderson 1998). The good linear relationship between the logarithm of the ET rate with the reduction potential of the donor is evidence (O'Neill and Chapman 1985), but not proof [cf. Jagannadham and Steenken 1988a; Steenken 1988] that this reaction proceeds *via* an outer-sphere ET. At high pH, when $G^{\bullet}$ is deprotonated, poorer reductants are no longer capable of reducing this intermediate (O'Neill and Chapman 1985). The reaction of $^{\bullet}G$ with NO_2^- is noticeably slower [$k = 2.6 \times 10^6$ dm^3 mol^{-1} s^{-1}; Shafirovich et al. 2001] than the above reactions which are close to diffusion controlled. Yet, cellular levels of NO_2^- that are normally $<2 \times 10^{-4}$ mol dm^{-3} may be increased to $>1 \times 10^{-3}$ mol dm^{-3} under certain conditions. The product of this reaction, $^{\bullet}NO_2$, oxidizes 8-oxo-G (see below).

The fate of $G^{\bullet}$ in the absence of any additive is as yet unknown. It decays bimolecularly with a rate constant of some 10^8 dm^3 mol^{-1} s^{-1} (Faraggi et al. 1996), but there is increasing evidence that in competition, at least at elevated pH [$pK_a(G^{\bullet}) = 10.8$], its radical anion also decays unimolecularly [$k = 5 \times 10^3$ s^{-1} at pH 11 in the case of Guo and dGuo; Faraggi and Klapper 1994; Faraggi et al. 1996]. The nature of this unimolecular transformation is as yet unknown.

Unless a water-assisted disproportionation (cf. Wang et al. 1996) or a disproportionation by ET takes place, $G^{\bullet}$ has to dimerize. These dimers have escaped identification, but the various canonical mesomeric forms shown below indicate

that there can be quite a number of potential dimers. A potential product of the disproportionation is 8-oxo-G. Its yield in •OH-induced reactions is that low that this process must be of minor importance (see below).



Mesomeric forms of G•

G• does not react with O_2 on the pulse radiolysis time scale due to its high spin density at heteroatoms (von Sonntag 1994). This is further supported by the observation that even at the low dose rates of γ-radiolysis there is very little O_2-uptake [$G(-O_2) = 0.7 \times 10^{-7}$ mol J^{-1}] when G• is generated by Tl(II) in N_2O/O_2-saturated solutions (Al-Sheikhly 1994). From these data, the rate contant of G• with O_2 must be $\leq 10^2$ dm^3 mol^{-1} s^{-1}.

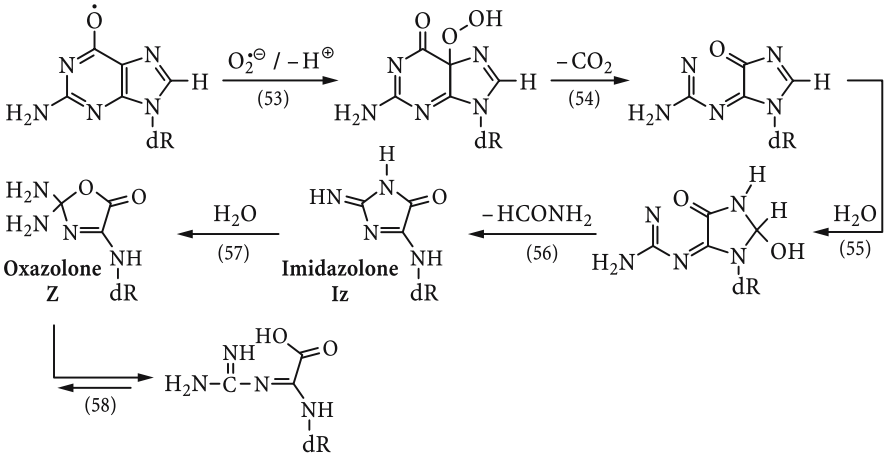
An important dGuo oxidation product is imidazolone (Iz), which decomposes into oxazolone (Z) and/or its ring-open guanodino acid form (Ravanat et al. 1991; Raoul et al. 1996; Gasparutto et al. 1999; for kinetic data see Table 10.5). At high pH, these compounds release guanidine for which an assay is available (Kobayashi et al. 1987) (Chap 13.3).

The proposed (Buchko et al. 1993; Cadet et al. 1994; Raoul et al. 1996) mechanism of the guanidine-releasing products formed in the •OH-induced (30% of •OH yield in the γ-radiolysis of air-saturated dGuo solutions; Douki and Cadet 1996), benzophenone- and riboflavin-sensitized oxidation of dGuo and also in the case of the oxidation of DNA by *tert*-butoxyl radicals (Adam et al. 1998) requires the reaction of $G^{\bullet+}/G^{\bullet}$ with O_2. This is in poor agreement with the data mentioned above. In fact, the formation of guanidine releasing products is due to a reaction of G• and $O_2^{\bullet-}$ [reactions (53)-(58); Misiaszek et al. 2004; in competition with ET that reformes G; see also Chap. 12.3]. On the nucleoside level, reaction (53) is very fast [$k = 4.4 \times 10^9$ dm^3 mol^{-1} s^{-1}; von Sonntag 1994], while it is an order of magnitude slower in ss- and dsODNs (Misiaszek et al. 2004). This drop may be due to an electrostatic repulsion of $O_2^{\bullet-}$ in the case of the negatively charged ODNs.

Table 10.5. Half-lives of Iz, the Z and 8-oxo-G at various conditions of temperature and pH (Raoul et al. 1996)

pH	Temp / °C	Iz	Z	8-oxo-G
7	20	24 h	Stable	Stable
	37	147 min	Stable	Stable
10	65	5.0 (11.2)[a] min	21.8 min	Stable
13	20	68.6 min	20.2 (58.5)[a] min	n.d.
	37	26.7 min	5.1 (23.8)[a] min	n.d.
	65	3.1 min	3.3 min	38.6 min

n.d., Not determined.
[a] Second component



Not all photooxidation pathways can be attributed to radical cations as the only intermediate. For example, in the benzophenone-sensitized reaction, some and β→α and α→β isomerization (in the case of α-dGuo) is observed (Vialas et al. 1999). while no such isomerization occurs upon the Mn-TMPyP-mediated oxidation by $KHSO_5$ (Vialas et al. 1999). Here, Iz is the only detected product [90% yield; Vialas et al. 1998] which points to different intermediates in these two systems. Ascorbate or cysteine, may intercept G•, and in their presence the oxazolone yield is strongly reduced (Douki et al. 1999).

The redox potential of A• is higher by 0.27 V than that of G• (Table 10.2), and thus reductants capable of reducing G• also reduce A• (Jiang et al. 1999a). In ODNs, it also undergoes ET from a neighboring G (Bamatraf et al. 2000).

Table 10.6. Compilation of rate constants for the reactions of $^{\bullet}OH$, $H^{\bullet}$ and e_{aq}^{-} with the nucleobases and related compounds. (Buxton et al. 1988)

Substrate	$^{\bullet}OH$	$H^{\bullet}$	e_{aq}^{-}
Ade	6.1×10^9	1.0×10^8	9.0×10^9
dAdo	4.6×10^9	–	8.2×10^9
Ado	5.8×10^9	2.0×10^8	1.1×10^{10}
AMP	4.1×10^9	1.9×10^8	3.8×10^9
Cyt	6.3×10^9	9.2×10^7	1.3×10^{10}
dCyd	6.0×10^9	–	–
Cyd	5.8×10^9	–	1.3×10^{10}
Gua	–	–	1.4×10^{10}
Gua anion	9.2×10^9	–	2.0×10^9
dGuo	4.1×10^{9a}	–	1.7×10^{10}
dGMP	4.7×10^9	–	6.0×10^9
Guo	7.8×10^9	5.0×10^8	6.0×10^9
Thy	6.4×10^9	6.8×10^8	1.8×10^{10}
Thd	4.7×10^9	3.2×10^8	–
Ura	5.7×10^9	4.7×10^8	1.5×10^{10}
Urd	5.2×10^9	–	1.4×10^{10}

[a] Steenken (1989)

10.3 Reactions Induced by $^{\bullet}OH/O^{\bullet-}$ and $^{\bullet}H$

10.3.1 Rate Constants

The nucleobases and related compounds react with $^{\bullet}OH$ at close to diffusion-controlled rates. A compilation of rate constants is given in Table 10.6. In nucleosides and nucleotides, $^{\bullet}OH$ attacks mainly at the base moiety, but some H-abstraction also occurs at the sugar moiety (Chap. 3.3). It is recalled that the high reactivity of $^{\bullet}OH$ results in a very low $^{\bullet}OH$ steady-state concentration, and reactions with substrates, even when present at rather low concentrations, predominate over the their reactions with $^{\bullet}OH$-induced substrate radicals. Thus,

Table 10.7. Reactions of $^{\bullet}OH$ with some pyrimidines. Yields of addition at *C*(5) and *C*(6). H-abstraction where applicable. (Fujita and Steenken 1981; Al-Sheikhly and von Sonntag 1983; Hazra and Steenken 1983)

Pyrimidine	Addition at C(5)	Addition at (C6)	H-Abstraction
Ura	82	18	–
Thy	60	30	10
6MeUra	88	12	Little
Isoorotic acid	63	37	–
Orotic acid	86	14	–
$1,3Me_2Ura$	78	17	Little
poly(U)	70	23	7
Cyt	87	10	–
1MeCyt	87	8	–
2MeCyt	92	10	–
5MeCyt	65	22	13
5CarboxyCyt	82	24	–

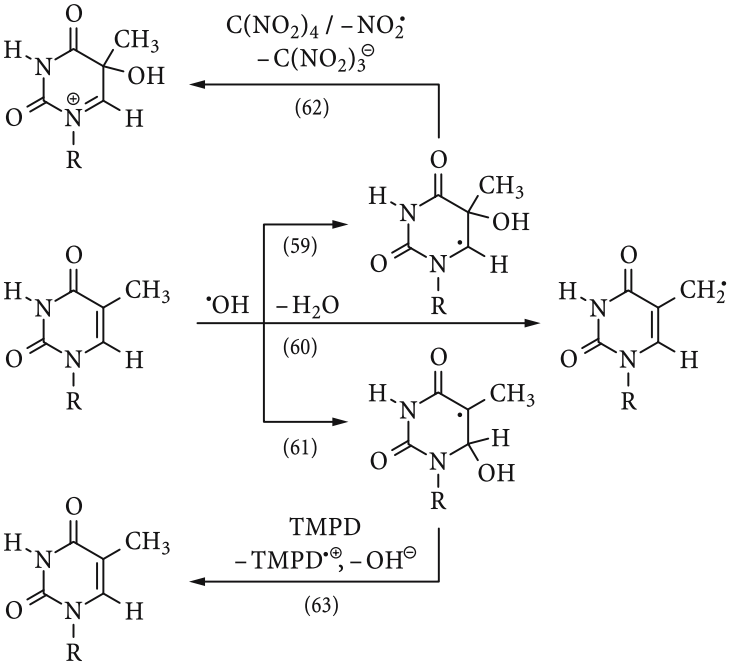
mechanisms involving consecutive additions of two $^{\bullet}OH$ that still continue to be considered in the literature [e.g., Jaussaud et al. 2000] have to be rejected.

The basic form of $^{\bullet}OH$, $O^{\bullet-}$ [$pK_a(^{\bullet}OH) = 11.8$], reacts much more slowly with the nucleobases (Ioele et al. 1998) that are all deprotonated at the pH where $O^{\bullet-}$ predominates (Table 10.11). It will be shown below that $^{\bullet}OH$ and $O^{\bullet-}$ differ substantially in their reactions.

10.3.2 Determination of the Site of $^{\bullet}OH$-Attack

Pyrimidines. When $^{\bullet}OH$ reacts with the pyrimidines, it adds to the *C*(5)–*C*(6) double bond, and in the case of Thy it also abstracts to a minor extent an H-atom from the methyl group [reactions (59)–(61)]. A radical at *C*(6) is formed upon addition at *C*(5) [reaction (59); for EPR studies see, e.g., Hildenbrand et al. 1989; Schulte-Frohlinde and Hildenbrand 1989; Catterall et al. 1992; for DFT calculations, see Wetmore et al. 1998b]. This radical has reducing properties due to the interaction with the electron pair at the neighboring nitrogen, and in a pulse radiolysis experiment its yield can be determined by its rapid reaction with TNM which yields the strongly absorbing nitroform anion [reaction (62); $\varepsilon(350nm) = 15{,}000\ dm^3\ mol^{-1}\ cm^{-1}$].

On the other hand, the *C*(6)-•OH-adduct formed in reaction (61) has oxidizing properties (note its mesomeric form with the radical at oxygen; quantum-mechanical calculations indicate that it is the most oxidizing radical among all conceivable •OH- and •H-adducts of the nucleobases; Colson and Sevilla 1995). The yield of this oxidizing radical can be determined with the help of a strong reductant such as TMPD [reaction (63); the TMPD radical cation is monitored; ε(565 nm) = 12,500 $dm^3\ mol^{-1}\ cm^{-1}$].



The allylic radical formed upon H-abstraction from the methyl group [reaction (60)] has neither reducing nor oxidizing properties, and its yield may be deducted from the difference of the sum of reducing and oxidizing radicals with respect to the total •OH yield. A series of such experiments has been carried out (Fujita and Steenken 1981; Al-Sheikhly and von Sonntag 1983; Hazra and Steenken 1983), and their results are compiled in Table 10.7.

The yield of 1,4-benzosemiquinone anions in the presence of 1,4-benzosemiquinone (Hayon and Simic 1973; Simic and Hayon 1973; Bamatraf et al. 1998) has been taken as a measure for the yield of reducing radicals formed upon •OH attack. The values reported for Cyt (83% by Bamatraf et al. 1998, 75% by Simic and Hayon 1973) and Thy (63% by Bamatraf et al. 1998, 35% by Simic and Hayon 1973, 41% by Hayon and Simic 1973) do not agree well with one another and only approximately with the data in Table 10.7. The values for dCyd (75%) and Thd (47%) (Bamatraf et al. 1998) are surprisingly low in comparison, especially since nearly all sugar derived radicals should also react with 1,4-benzoquinone by reduction. However, in this context it is recalled that an addition which is the more

Table 10.8. π-Electron densities of the HOMO in some pyrimidines at the relevant carbons as calculated by the DFT B3LYP/6-31G(d)//SCRF=PCM method in water (Naumov and von Sonntag, unpublished results). Values of the percentage of •OH-attack (in parentheses) are taken from Table 10.7

Nucleobase	C(5)	C(6)
Thy	0.204 (60%)	0.082 (30%)
Ura	0.222 (82%)	0.063 (18%)
6MeUra	0.236 (88%)	0.062 (12%)
Cyt	0.205 (87%)	0.045 (10%)

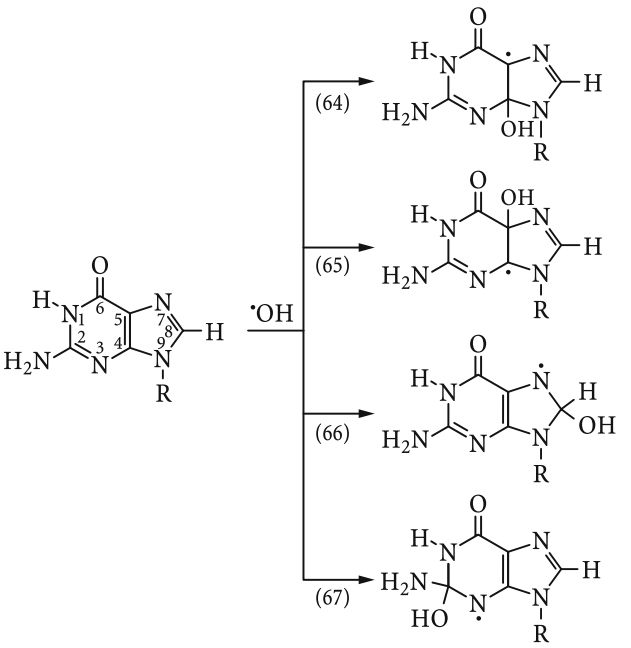
general reaction of radicals with 1,4-benzoquinone (Veltwisch and Asmus 1982; Schuchmann et al. 1998), is also given, at least to some extent, by other reducing radicals such as •CH_2OH (Simic and Hayon 1973; von Sonntag et al. 2004).

The rate of the oxidation of the reducing *C*(5)-•OH-adduct by Cu(II) varies between 10^6 dm^3 mol^{-1} s^{-1} and 10^8 dm^3 mol^{-1} s^{-1} depending on the complexing agent (Chabita et al. 1996; Fujita et al. 1996).

The strongly electrophilic •OH is very regioselective when adding to polarized C–C double bonds. The reaction is goverened by kinetics rather than thermodynamics. For the H-abstraction reaction (60), ΔG_{aq} = -112.2 kJ mol^{-1} has been calculated, while for the addition reactions (59) and (61) -42.6 kJ mol^{-1} and -85.5 kJ mol^{-1}, respectively, were obtained (Wu et al. 2004). As is seen from Table 10.7, the preference for •OH-attack does not correlate with the exothermicity of the reaction. In Table 10.8, calculated electron densities are given. In the pyrimidine series, they reflect well the experimental data obtained by redox titration (Table 10.7). In the purine series (see below), the situation seems to be more complex. The π-electron density does not sufficiently reflect the preferred site of attack, and more elaborate calculations that take the Mulliken charge into consideration as well are required (Naumov and von Sonntag, unpubl. results).

Purines. In general, •OH readily adds to double bonds but undergoes ET reactions only very reluctantly (Chap. 3.2). This also applies to purines despite their relatively low reduction potentials (Table 10.2). Thus, G• which is formed in the reaction of •OH with dGuo has a short-lived •OH-adduct rather than $G^{\bullet+}$ as precursor (Candeias and Steenken 2000), and the H-abstraction that could also lead to G• (for theoretical calculations see Mundy et al. 2002) does not occur to any significant extent.

The reactions of •OH with dGuo are shown in reactions (64)–(67).

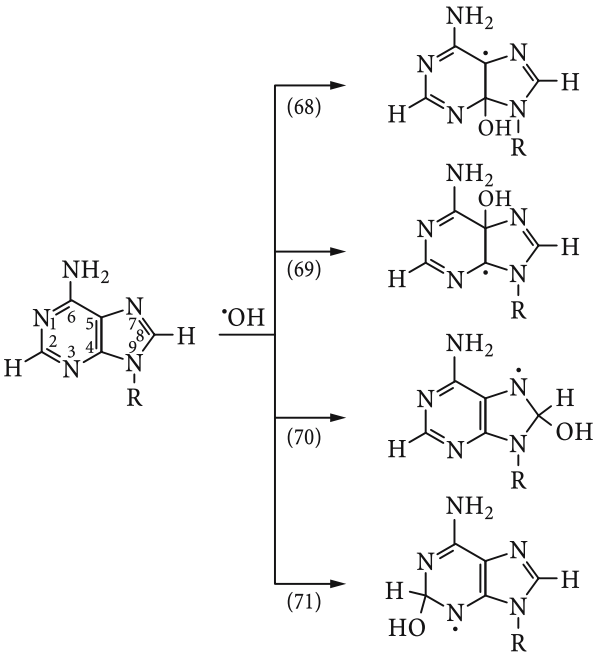


Of these radicals, 60–70% have oxidizing properties (for the reactions of purine-•OH-adducts with a number of reductants see, e.g., O'Neill 1983, 1984; O'Neill et al. 1985; Shi et al. 1999a; Candeias and Steenken 2000). This includes the •OH-adducts that eliminate rapidly water yielding G• (Steenken 1989). Its main precursor has been has been suggested to be the *C*(4)-•OH-adduct (~60-70%; Vieira et al. 1993; Candeias and Steenken 2000). This radical eliminates water leading to the even more strongly oxidizing G• [cf. reaction (77)].

The *C*(8)-•OH-adduct has reducing properties, and from its rapid reaction with $Fe(CN)_6^{3-}$ or methylviologen its yield has been determined at 17% (Candeias and Steenken 2000). It is the precursor of FAPY-dGuo and 8-oxo-dGuo whose combined yields have been determined after radiolysis in N_2O-saturated solutions at ~10% (Berger and Cadet 1985; lower limit for the *C*(8)-•OH-adduct). The maximum yield of 8-oxo-dGuo in the presence of $Fe(CN)_6^{3-}$ as oxidant and in the acid pH range has been found to be 23% (von Sonntag and Schuchmann 2001). This must be taken as an upper limit, since under these conditions reaction (51) may contribute to the formation of the *C*(8)-•OH-adduct.

The *C*(2)-•OH-adduct is likely to eliminate ammonia, and from the low ammonia yield it seems not to exceed 1.5% (von Sonntag and Schuchmann 2001). With dGuo, some H-abstraction occurs also at the sugar moiety. Among others, this is indicated by an isomerization of dGuo (in low yields, see Table 10.13). Nearly all sugar-derived radicals must have reducing properties, and, based on the pulse radiolysis data mentioned above, the upper limit of their yield must be much less than the total of 17%.

The reactions of •OH with dAdo/Ado are depicted in reactions (68)–(71).

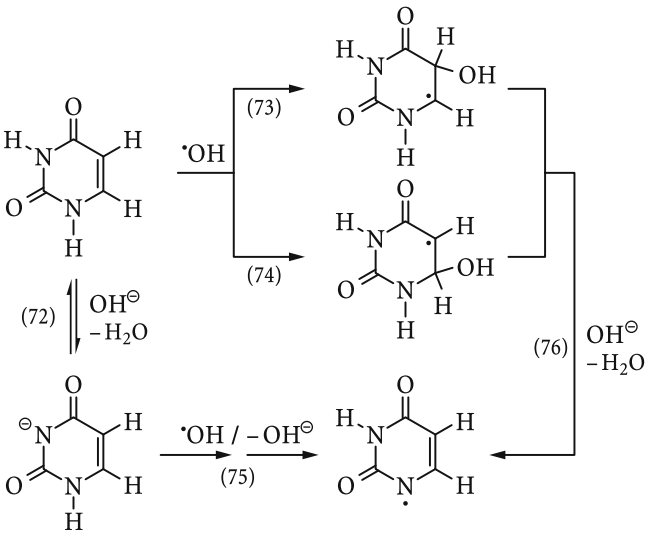


With Ado, only 32% of the radicals have oxidizing properties (O'Neill et al. 1985). It has been suggested that 37% add to the *C*(8) position [reaction (70)] and 50% to the *C*(4) position [reaction (68)] and <5% to *C*(5) [reaction (69)] with some H-abstraction at the sugar moiety (Vieira and Steenken 1990; Vieira et al. 1993).

The yield of 8-oxo-Ade from Ade has been reported to be the same in the absence of O_2 or in air-saturated solution, but is increased in the presence of small amounts of $Fe(CN)_6^{3-}$ (saturation level already at 4×10^{-6} mol dm^{-3}; Dias and Vieira 1997a,b), putting a question mark behind the suggestion (Vieira and Steenken 1991; Dias and Vieira 1997a) that in the presence of O_2 this product is formed by the elimination of $O_2^{\bullet -}$ from the *C*(8)-·OH-adduct peroxyl radical.

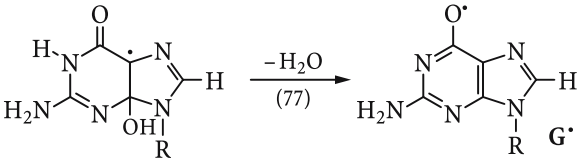
10.3.3 Water Elimination and Rearrangements

In basic solution, Ura and Thy undergo a series of reactions as depicted in reactions (72)–(76) for Ura as an example (Fujita and Steenken 1981). Ura dissociates at high pH [equilibrium (72); for pK_a values see Table 10.11]. Its ·OH-adducts can also be deprotonated at nitrogen leading to an oxidizing heteroatom-centered radical [reaction (76)].



Cyt has no pK_a in the basic pH range below 12 (Table 10.1). A number of transformations at pH <11 were observed by pulse radiolysis (Hissung and von Sonntag 1978). In Cyt and 5MeCyt, there is an OH^--induced reaction ($k = 1.4 \times 10^8$ dm^3 mol^{-1} s^{-1}) that is not observed with dCyd, indicating that the *N*(1)-H must be involved in this reaction, but the rate of reaction is somewhat too slow for a deprotonation at a heteroatom. EPR and product data that could shed some light on these reactions are missing. It is, hence, premature to suggest mechanistic details.

The *C*(4)-•OH-adduct of dGuo has oxidizing properties (note the mesomeric form with spin density at *O*(6)). It eliminates water yielding the even more strongly oxidizing G• [reaction (77); $k = 6 \times 10^3$ s^{-1} in the absence of buffer; Candeias and Steenken 2000].



A similar reaction has been suggested for the of dAdo that gives rise to A• [reaction (78); $k = 1.9 \times 10^4$ s^{-1}]. Again, the *C*(4)-•OH-adduct is less oxidizing than A• and reacts with TMPD with 2.3×10^7 dm^3 mol^{-1} s^{-1}, while A• reacts with 2×10^9 dm^3 mol^{-1} s^{-1} (Vieira and Steenken 1990).

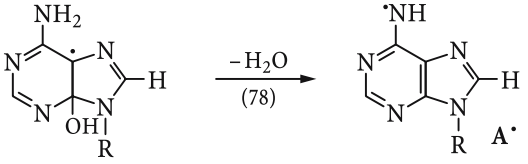


Table 10.9. γ-Radiolysis of N_2O-saturated aqueous solutions of Ura. Products and their *G* values (unit: 10^{-7} mol J^{-1}) at different pH values. (Idris Ali and Scholes 1980)

Product	pH 2	pH 4	pH 6	pH 8
Dimers	1.35	3.3	4.38	1.65
5,6-Dihydro-5,6-dihydroxyuracil (Ug; *cis* and *trans*)	0.68	0.32	0.30	0.18
5,6-Dihydro-5(and 6)-hydroxyuracil	0.31	0.44	0.29	0.18
Isobarbituric acid	0.29	0.12	0.52	0.16
Ura consumption	2.68	4.25	5.51	2.11

10.3.4 Products in the Absence of Oxygen

Pyrimidines. Detailed studies concerning the products that are formed upon $^{\bullet}OH$-attack (radiolysis of N_2O-saturated solutions) are available for Ura, Thy, Cyt and 1,3Me_2Ura (Tables 10.9-10.12).

In addition, the radiolysis of Ura in deoxygenated solutions (in the absence of N_2O) has also found attention (Infante et al. 1974; Shragge et al. 1974). Under such conditions, however, not only the $^{\bullet}OH$-induced reactions play a role, but also the electron-adduct radical with all the ensuing mechanistic complications contributes to the products.

The *N*(1)-H of the nucleobases can take part in disproportionation reactions leading to the formation of isopyrimidines (for their reactions see below). This crucial hydrogen is not available in nucleosides which are a much closer model for DNA. No detailed studies are available for pyrimidine nucleosides so far. A compound which has no hydrogen at *N*(1) is 1,3Me_2Ura. It still shows the major aspects of the base moiety of Ura- and Thy-containing nucleosides but is much more easy to handle analytically than nucleosides. Thus, results from a study on the γ-radiolysis of 1,3Me_2Ura in N_2O-saturated solutions (Table 10.12) may be a good guide for the reactions that one might have to envisage in the nucleosides and also in DNA. As has been discussed before, $^{\bullet}OH$ adds mainly at *C*(5) (~78%) and to a lesser extent at *C*(6) (~17%). Some H-abstraction at the methyls occurs as well. The major reaction of the dominating *C*(6)-yl radical is its recombination [reaction (79)].

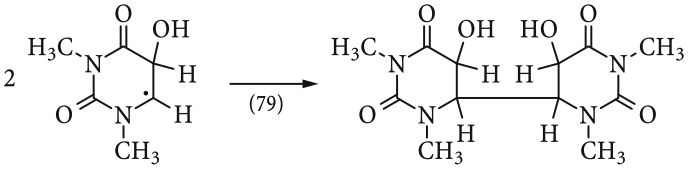


Table 10.10. γ-Radiolysis of N_2O-saturated aqueous solutions of Thy. Products and their *G* values (unit: 10^{-7} mol J^{-1}). *A* Infante et al. (1973), *B* Nishimoto et al. (1983a)

Product	A	B
5,6-Dihydro-5,6-dihydroxythymine (Tg; *cis* and *trans*)	2.4	0.35
5-Hydroxy-5,6-dihydrothymine	–	0.09
6-Hydroxy-5,6-dihydrothymine	0.15	0.1
5,6-Dihydrothymine	0.1	0.2
5-Hydroxymethyluacil	0.25	0.3
5-Hydroxy-5-methylbarbituric acid	0.11	–
5-Methylbarbituric acid	–	0.06
Formylpyruvylurea	0.12	–
Dimers	0.27	n.d.
Unknown	0.11	–
Thy consumption	4.05	2.8

n.d., Not determined

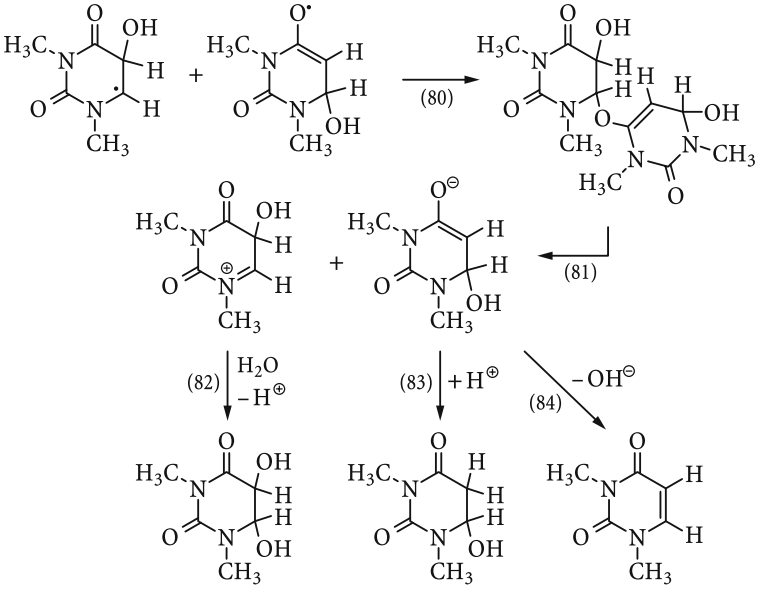
Table 10.11. γ-Radiolysis of N_2O-saturated aqueous solutions of Cyt. Products and their *G* values. (Dizdaroglu and Simic 1984b)

Product	G/10^{-7} mol J^{-1}
Dimers	3.3
5-Hydroxycytosine	1.5
5,6-Dihydroxycytosine	0.2
5,6-Dihydro-5,6-dihydroxyuracil (Ug)	0.15
6-Hydroxycytosine	0.08
5,6-Dihydro-5,6-dihydroxycytosine (Cg)	0.05
Ura	0.02
Cyt consumption	5.8

Table 10.12. γ-Radiolysis of N_2O-saturated aqueous solutions of 1,3Me_2Ura. Products and their *G* values (unit: 10^{-7} mol J^{-1}; Al-Sheikhly and von Sonntag 1983)

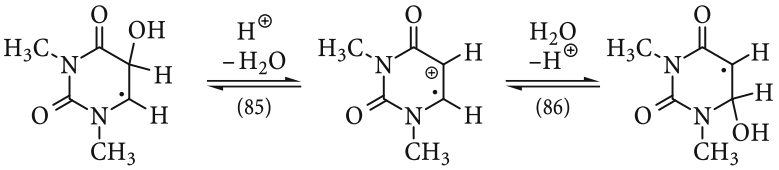
Product	pH 3	pH 6.5	pH 10.4
5,6-Dihydro-5-hydroxy-1,3-dimethyluracil	0.4	0.8	0.6
5,6-Dihydro-6-hydroxy-1,3-dimethyluracil	<0.1	0.2	<0.1
1,3-Dimethylisobarbituric acid	0.15	0.1	≤0.1
5,6-Dihydro-5,6-dihydroxy-1,3-dimethyluracil	1.6	0.9	0.85
Dimers (of 6-yl radicals, in monomer units)	1.8	3.5	3.3
Dimers (involving H-adduct radicals)	<0.1	0.2	<0.1
1,3Me_2Ura consumption	4.0	5.9	5.3

In competition, the C(6)-yl and C(5)-yl radicals may disproportionate, possibly via an adduct [reactions (80) and (81)]. This yields the hydrate via an enol [reaction (83)]. The other product is the glycol [reaction (82)]. In the original paper (Al-Sheikhly and von Sonntag 1983), it has been proposed that it may be formed in an ET reaction. Due the considerable rearrangement energies involved in ET reactions as compared to radical recombination reactions, it is now considered that this ET reaction might occur via an addition/elimination process [reactions (80) and (81)] such as has also been found for other systems.



In this context, it should be mentioned that the glycols derived from Thd (Tg) are now also very well characterized (Jolibois et al. 1996).

As can be seen from Table 10.12, there are practically no changes in the product yields when neutral and basic solutions are compared. However, the yield of the dimers is drastically reduced in acid solutions, while that of the glycol is enhanced. Altogether, the *G* value of consumption is also reduced which points to an increase of the importance of disproportionation reactions. This has been explained by an acid-catalyzed transformation of the reducing *C*(6)-yl radical into the oxidizing *C*(5)-yl radical [reactions (85) and (86); Al-Sheikhly and von Sonntag 1983].

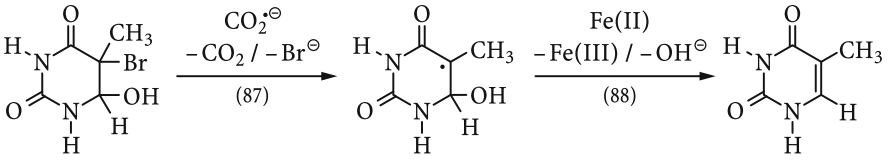


This interpretation indicates that the oxidizing radical is thermodynamically favored over the reducing radical. This is confirmed by quantum mechanical calculations (Naumov and von Sonntag, unpubl. results; Wu et al. 2004), and when low-temperature sulfuric acid glasses containing Ura or Thy are X-irradiated, the dominant radical seen by EPR is the 5-yl radical (Riederer and Hüttermann 1982).

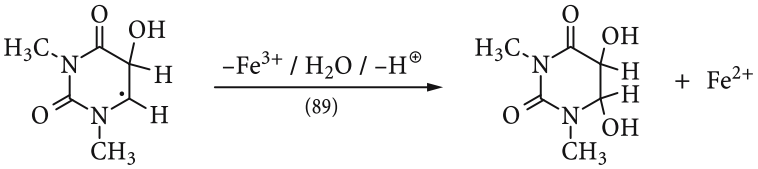
With d(TpA), a number of products were separated by HPLC, and two of them (the dinucleoside with a Tg lesion (four stereoisomers) and one with a 6-hydroxy-5,6-dihydrothymine lesion (two isomers)) were characterized by NMR (Belfi and Box 1985). Further identified products were Thy, Ade and dAMP. With d(GpC) and d(CpG) γ-irradiated in N_2O-saturated solutions, the corresponding Ug (deamination products of the Cg) and the 5- and 6-hydroxy dCyd substitution products were detected (Paul et al. 1987b). In addition, dinuclosides with intact dCyd and a 8-oxo-G moiety were observed. With d(TpA) and d(ApT), Tg, the free bases and the monophosphates were detected (Paul et al. 1987a).

The *C*(5)-$^{\bullet}$OH-adduct radicals of pyrimidines can be reduced by thiols (Barvian and Greenberg 1992) or by cylohexa-1,4-diene (Barvian et al. 1996). The rate of reduction by thiols seems to be slow. Otherwise, the formation of thioethers (the recombination product of a cysteamine-derived thiyl radical with a TMP-derived $^{\bullet}$OH-adduct; $G = 1.55 \times 10^{-7}$ mol J^{-1}) in the radiolysis of N_2O-saturated solution of TMP in the presence of 5% cysteamine (Grachev et al. 1983, 1986) are difficult to explain.

The UV spectrum of the (oxidizing) *C*(6)-$^{\bullet}$OH-adduct of Thy has been obtained (Deeble and von Sonntag 1985) and its reactions were studied (Nishimoto et al. 1983c) by generating it specifically via the reaction of 5-bromo-6-hydroxythymine with $CO_2^{\bullet-}$ [reaction (87)]. In the presence of transition metal ions in their low oxidation states and with the more reducing ones such as Fe(II), Pt(II) or Cu(I), Thy in high yields [reaction (88)] and only traces of 6-hydroxy-5,6-dihydrothymine were observed. This indicated that a potential enolate must eliminate an OH^- rather than protonates at *C*(5) (ketonization).



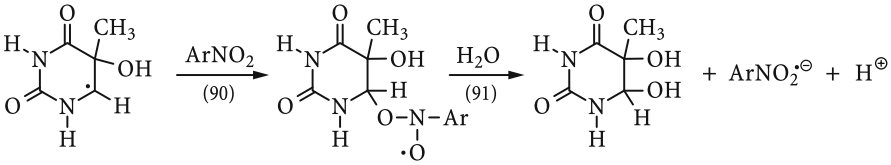
When •OH is generated by the Fenton reaction, i.e. in the presence of H_2O_2 and Fe^{2+}/Fe^{3+}, the transition metal ions (and possibly H_2O_2; in fact, photolysis of H_2O_2 in the presence of Ura and Thy is a very convenient way of preparing their glycols in good preparative yields; Hahn and Wang 1977) undergo redox reactions with the radicals that are formed by •OH-attack. In the case of 1,3Me_2Ura, the otherwise dominating product, the dimers (Table 10.12), are no longer formed. Instead, the 1,3Me_2Ura glycol is now practically the only product (Theruvathu et al. 2001). It is formed in a short chain reaction; 2.7 mol 1,3Me_2Ura are consumed and glycol formed for 1 mol of Fe^{2+} consumed. The major radical, the reducing *C*(5)-•OH-adduct is oxidized by Fe^{3+} [reaction (89)] thereby regenerating Fe^{2+} which give rise to further •OH upon its reaction with H_2O_2 (Chap. 2.5).



The glycol is also the most important product when Ura is γ-irradiated in aqueous solution in the presence of Fe^{3+} (Bhattacharyya and Mandal 1983).

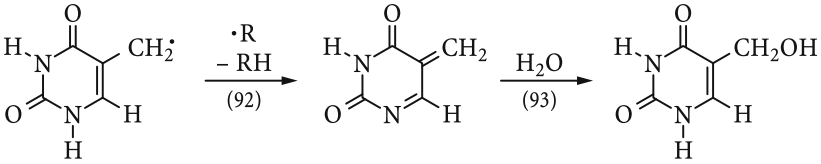
In •OH-induced reactions of Thy, Tg and 5-hydroxymethyluracil yields are markedly enhanced in the presence of nitroxyl radicals such as TEMPO (Kagiya et al. 1983). Adducts may be the intermediates. In the case of the nitroxyl radical TAN and for the Thd system, such adducts have been characterized (Berger et al. 1985).

The Tg yield is also substantially enhanced in the presence of electron-affinic sensitizers, and in the radiolysis of N_2O-saturated solutions its yield has been reported to increase from $G = 0.32 \times 10^{-7}$ mol J^{-1} in the absence to $G = 1.6 \times 10^{-7}$ mol J^{-1} in the presence of misonidazole (Nishimoto et al. 1983a). It has been emphasized that electron-afficinic sensitizers do not necessarily undergo an outer-sphere ET, but may react by addition (Wardman 1987). This type of reaction has been investigated in detail using nitroaromatics as oxidants (Jagannadham and Steenken 1984, 1988a,b; Steenken and Jagannadham 1985). When the adducts hydrolyze (possibly in competition with other reactions, since the Tg yield depends on the oxidant; Nishimoto et al. 1983a), the products are identical to the ones expected to be formed by ET [reactions (90) and (91)].



When an *N*(1)-H is avalable as in free-base systems, deprotonation at *N*(1) speeds up the hydrolysis (Steenken and Jagannadham 1985). For example, the corresponding *p*-nitroacetophenone Ura adduct decays with $2.4 \times 10^5\ s^{-1}$ when deprotonated at *N*(1). The *N*(1)-alkylated pyrimidines also hydrolyze, but slower (e.g., $k = 4.5 \times 10^3\ s^{-1}$ in the case of uridylic acid) when deprotonated at *N*(3). In neutral solution, the rate of hydrolysis must be considerably slower, possibly that slow that other reactions may compete.

It has been mentioned above that •OH abstracts from Thy also to a minor extent a hydrogen at methyl. Indeed, 5-hydroxymethyluracil has been detected to be formed in about 5% yield (Nishimoto et al. 1983a). It is suggested here that the introduction of the OH group may have occurred via an isopyrimidine-type intermediate as depicted in reactions (92) and (93).



It is interesting that in the presence of misonidazole the 5-hydroxymethyluracil yield is reduced to one quarter (Nishimoto et al. 1983a). Addition of misonidazole to the allylic radical which has no marked reductive power (note that this radical is also not oxidized by the more reactive oxidant TNM, see above) must thus give products other than 5-hydroxymethyluracil.

In the case of Cyt, the expected glycols, Cg, have only been detected in low amounts; instead, major amounts of 5OHCyt and the deamination product, Ug, were reported (Table 10.11, see also Table 10.16). The dCyd glycols are now, however, well characterized (Tremblay et al. 1999). They readily undergo water elimination ($t_{1/2}$ = 50 min at 37 °C). This may have been the reason, why in earlier studies only low yields of these products were detected. It may be mentioned here, that in DNA the lifetime of the Cg lesion is much longer ($t_{1/2}$ = 20 h), and thus this product stands a good chance to be involved in the repair processes.

In the reaction of Cyt (Mandal and Yamamoto 1985) and dCyd (Yamamoto and Mandal 1988) with •OH fluorescent products are formed; their chemical structures are as yet unknown.

Purines. Product studies on the reactions of •OH with the purines are not at the same level as those of the pyrimidine series. Only the products that can be traced back to an •OH-attack at *C*(8) and involve the sugar moiety were detected with the presently available analytical techniques. Yet, •OH attack at *C*(8) is only around 17% in Guo/dGuo and ~30% in Ado/dAdo (see above). Attack at the

Table 10.13. γ-Radiolysis of dGuo (5×10^{-3} mol dm^{-3}) in deaerated and N_2O-saturated solutions. Products and their *G* values (unit: 10^{-7} mol J^{-1}; Berger and Cadet 1985)

Product	N_2	N_2O
N^6-(2′-Deoxy-α-D-*erythro*-pentopyranosyl)-2,6-diamino-5-formamidopyrimid-4-one (α-FAPY-G)	0.27	0.26
N^6-(2′-Deoxy-β-D-*erythro*-pentopyranosyl)-2,6-diamino-5-formamidopyrimid-4-one (β-FAPY-G)	0.09	0.1
5′-8-Cyclo-2′,5′-dideoxyguanosine	0.05	0.06
8-oxo-G	–	0.25
Gua	0.2	0.4
9-(2′-Deoxy-α-D-*erythro*-pentopyranosyl)guanine	0.02	0.03
9-(2′-Deoxy-β-D-*erythro*-pentopyranosyl)guanine	0.01	0.02
9-(2′-Deoxy-α-D-*erythro*-pentofuranosyl)guanine	0.02	0.02
9-(2′-Deoxy-α-L-*threo*-pentofuranosyl)guanine	0.02	0.02
9-(2′-Deoxy-β-D-*erythro*-pento-1,5-dialdo-1,4-pyranosyl)-guanine	0.07	0.08
2-Deoxyribonolactone, 2-dRL	[a]	[a]
dGuo consumption	0.83	1.6

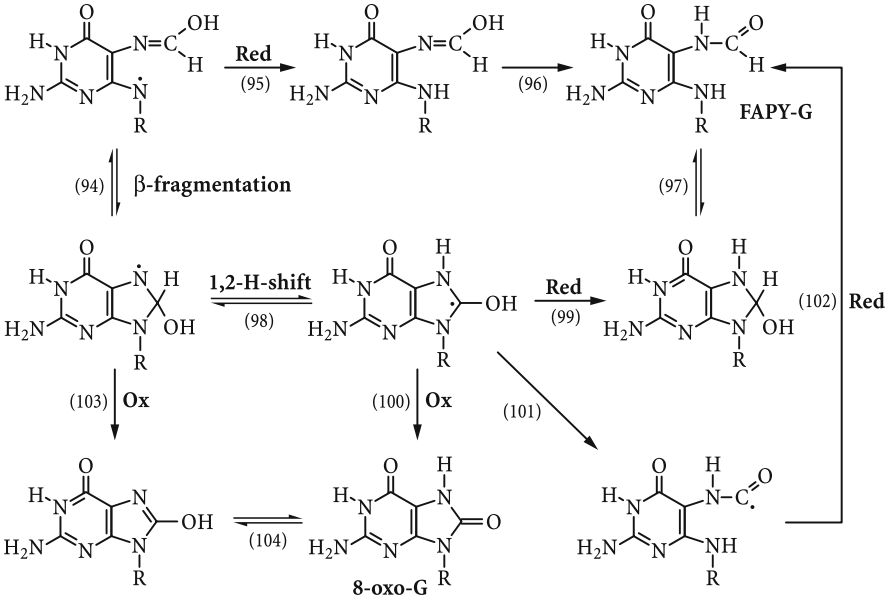
[a] Observed, but not quantified

Table 10.14. γ-Radiolysis of Ade (2×10^{-3} mol dm^{-3}) in N_2O-saturated solutions. Products and their *G* values (unit: 10^{-7} mol J^{-1}; van Hemmen and Bleichrodt 1971; see also van Hemmen 1971)

Product	*G* value
4,6-Diamino-5-formamidopyrimidine (FAPY-A)	0.2
8-Hydroxyadenine (8-oxo-A)	0.35
6-Amino-8-hydroxy-7,8-dihydropurine	0.1
Ade consumption	1.0

sugar moiety will also not exceed 10-15%. Product yields of the reaction of •OH with dGuo and Ade are compiled in Tables 10.13 and 10.14. It is re-emphasized that the material balance with respect to •OH formed under the given conditions is very poor.

Based on a pulse-radiolytic study, it has been concluded that 17% of •OH add to the *C*(8)-position of dGuo (see above). The ensuing reactions are complex and seem to involve a number of equilibria, β-fragmentation and 1,2-H-shift reactions as depicted in reactions (94)–(104).



Final products then arise from subsequent reduction/oxidation (H-transfer) reactions. The *C*(8)-•OH-adduct undergoes rapid ring-opening [reaction (94), $k = 2 \times 10^5\ s^{-1}$]. The *C*(8)-•OH-adduct and its ring-opened successor radical are reducing radicals and readily react with $Fe(CN)_6^{3-}$ or methylviologen. The oxidation product of the ring-opened radical has not yet been identified, but the oxidation product of the *C*(8)-•OH-adduct is a well-known product, 8-oxo-G. With 9MeGua, the formation of 8-oxo-9MeGua increases to about 20% in the presence of $Fe(CN)_6^{3-}$ [reactions (103) and (104); Candeias and Steenken 2000]. Interestingly, the $Fe(CN)_6^{3-}$ concentration required to oxidize fully the precursor radical is much lower than suggested by the rate constant assigned to the ring-opening process, and it has hence been assumed that reaction (94) must be reversible. The yield of 8-oxo-G depends also on the pH. In the presence of $Fe(CN)_6^{3-}$, the maximum yield of $G = 1.3 \times 10^{-7}$ mol J^{-1} is reached at an $Fe(CN)_6^{3-}$ concentration of 5×10^{-4} mol dm^{-3} in acid solution (von Sonntag and Schuchmann 2001; see also Vieira et al. 1993). This *G* value corresponds to the yield of the *C*(8)-•OH-adduct, i.e. there seem to be no side reactions that compete with this oxidation.

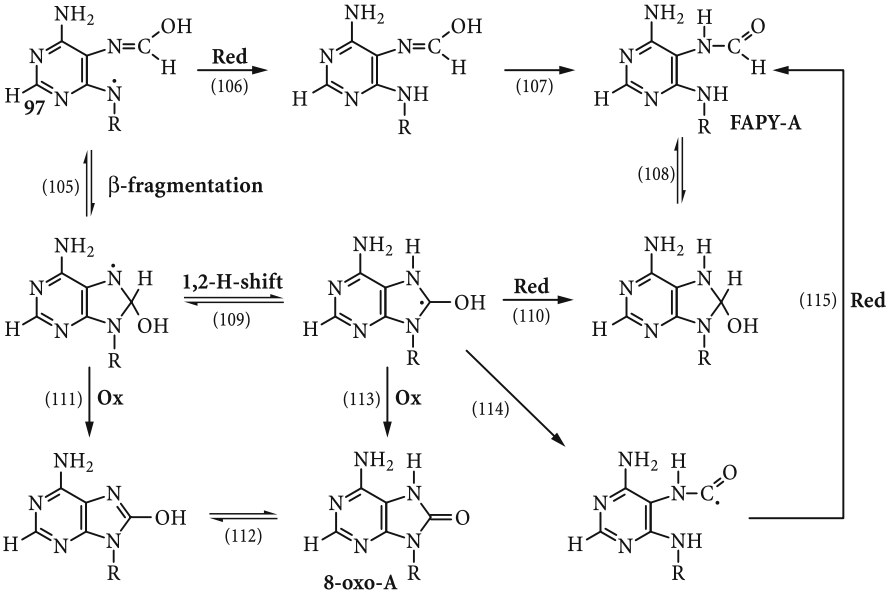
The ring-opened radical may also be reduced in, for example, disproportionation reactions to yield FAPY-G [reactions (95) and (96)]. A 1,2-H-shift [reac-

tion (98)], typical for heteroatom-centered radicals, may also contribute to the formation of 8-oxo-G [reaction (100)] and FAPY-G [reactions (99) and (97)]. In Table 10.13, the formation of two isomers is reported. With dAdo, considerable isomerization and even the hydrolysis of FAPY-A is observed (see below). Equilibrium (97) would not account for the isomerization of β-FAPY-G into α-FAPY-G. Thus it is likely that this isomerization occurred during work-up.

In this context it is worth mentioning that there is evidence (Candeias and Steenken 2000) that neither $G^{\bullet+}$ nor $G^{\bullet}$ react with water at an appreciable rate ($k = < 0.1\ s^{-1}$) forming the *C*(8)-•OH-adduct, that is, the precursor of 8-oxo-G. This seems to be in contradiction to a later suggestion by this group that at least the reaction of $G^{\bullet+}$ with water is highly exothermic (306 kJ mol^{-1}; Reynisson and Steenken 2002) and may undergo this reaction, [reaction (57)] quite readily unless kinetically disfavored.

8-oxo-G is typically synthesized according to Kasai and Nishimura (1984) by an Udenfriend-type reaction (Chap. 2.5), but in this synthesis a steady-state concentration of only about 6% (with respect to remaining dGuo) is reached (Wager, Schuchmann and von Sonntag, unpubl.). Mere competition for •OH would result in a much higher yield. 8-oxo-G is much more readily oxidized than dGuo itself (see above), and in the sequence of events there must be an oxidant other than •OH which is responsible for this low yield. Despite a more recent study of this reaction (Hofer 2001), mechanistic details remain unresolved.

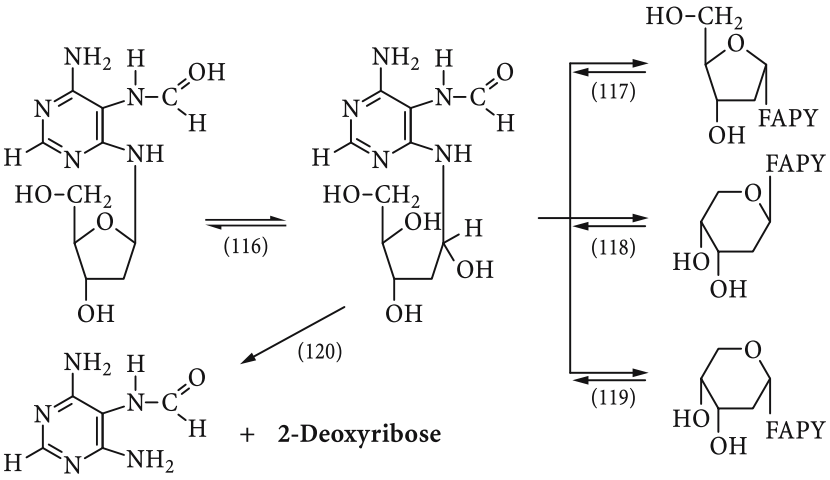
The *C*(8)-•OH-adduct of Ade and its derivatives shows the same reaction pattern [reactions (105)–(115)] as discussed above for Gua and its derivatives.



There are only minor differences. Its yield is higher (~30-37%). With N^6,N^6Me$_2$dAdo, the ring opening reaction (105) occurs at a rate of $9.5 \times 10^4\ s^{-1}$, and its rate is enhanced by OH^- (Vieira and Steenken 1987a,b). The yield of

FAPY-A has been found at 2% of •OH and that of 8-oxo-A at 5% (Vieira and Steenken 1990; Vieira et al. 1993). In the presence of $Fe(CN)_6^{3-}$, the 8-oxo-A yield is raised to 18% and that of FAPY-A is now < 0.2%. It is not yet understood why the balance (based on ~30% *C*(8)-•OH-adduct) is that poor.

FAPY-A is not stable in aqueous solution. It rapidly isomerizes and eventually is fully hydrolyzed [reactions (116)–(122); Raoul et al. 1995].



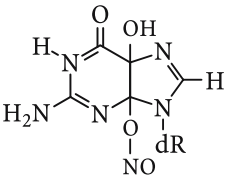
With FAPY-A derived from dAMP, where the pyranose forms cannot play a role, an anomeric ratio of $\beta/\alpha = 1.33$ develops after 6 h, while the deglycosylation half-life is 103 h at 37 °C (Greenberg et al. 2001). The deglycosylation of FAPY-G derived from dGMP is even slower.

The reactions of •OH-induced radicals of purine deoxyribonucleosides with nitroxyl radicals (for rate constants, see Brustad et al. 1972) have been studied with, for example, TAN, and it has been observed that the FAPY-products are no longer formed to a significant extent, and in the case of dAdo the formation of 8-oxo-A is enhanced (Berger and Cadet 1983b). This further supports the oxidizing properties of the nitroxyl radicals, although the formation of an adduct as an intermediate is very likely, considering that in the case of pyrimidine-derived radicals stable adducts have been observed (Cadet et al. 1979).

The attack of •OH at the *C*(2) position of dAdo is of little importance (see above), and it is thus not surprising that 2-hydroxy-dAdo is only a minor product (Frelon et al. 2002).

Fluorescent material is formed in the reaction of •OH with Ade, and it as been suggested that dimers may contribute to the as yet not fully characterized material (Yamamoto et al. 1985).

In the reaction of peroxynitrite with dGuo 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2′-deoxyguanosine has been reported to be formed (Douki et al. 1996) besides 8-oxo-G and Z (Douki and Cadet 1996) as well as 8-nitroguanine (Douki et al. 1996; Burney et al. 1999).



4,5-Dihydro-5-hydroxy-4-(nitrosooxy)-2'-deoxyguanosine

While 8-oxo-A is formed in the peroxynitite reaction with dAdo (Douki and Cadet 1996), 8-oxo-G (from dGuo) is not detected (Douki and Cadet 1996; Uppu et al. 1996) due to its much faster reaction with this reagent (Uppu et al. 1996).

Unless a non-radical mechanism accounts for the formation of 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2′-deoxyguanosine, $^{\bullet}NO_2$ must add to the 2′-deoxyguanosine $^{\bullet}OH$-adduct via its *O*-centered mesomeric form in contrast to the nitration of tyrosine, where $^{\bullet}NO_2$ reacts as an *N*-centered radical (van der Vliet et al. 1994; Lymar et al. 1999).

10.3.5 Products in the Presence of Oxygen

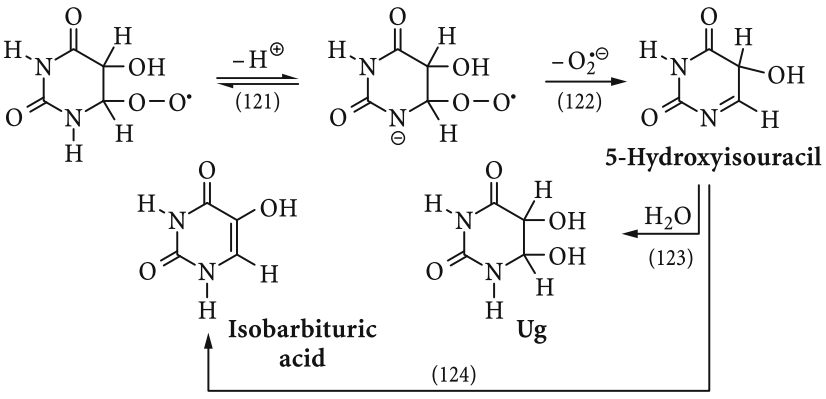
In the presence of O_2, most radicals are converted into the corresponding peroxyl radicals with the notable exception of heteroatom-centered radicals which do not react with O_2 at an appreciable rate (Chap. 8.2). However, even though peroxyl radical reactions may dominate in the reactions induced by the autoxidation of Fe(II)EDTA or Fe(II)NTA (Chap. 2.5), in the case of 2′-deoxynucleosides the subsequent reactions seem to be considerably modified by the presence of the transition metal ion, i.e. product ratios are found in these reactions which are different from those observed by ionizing radiation in the absence of Fe(II)/Fe(III) (Murata-Kamiya et al. 1998). A basis for understanding these differences may be the various redox reactions that the peroxyl radicals will undergo with Fe(II)/Fe(III) (cf. Yurkova et al. 1999; Theruvathu et al. 2001; see also Chaps 2.5 and 8.3).

Pyrimidines. The pyrimidine-$^{\bullet}OH$-adducts react with O_2 at close to diffusion-controlled rates yielding the corresponding peroxyl radicals ($k \approx 2 \times 10^9$ dm^3 mol^{-1} s^{-1}; Willson 1970). In basic solutions, but also in neutral solutions as long as the (dominating) *C*(6)-peroxyl radical has a sufficiently long lifetime, it undergoes $O_2^{\bullet-}$-elimination after deprotonation at *N*(1) [reactions (121) and (122)] (Schuchmann and von Sonntag 1983). Details of this kind of $O_2^{\bullet-}$-elimination, including the determination of the pK_a value of the peroxyl radical, has been studied for a very similar system, glycine anhydride (Mieden et al. 1993). In reaction (122) an isopyrimidine (5-hydroxyisouracil) is formed. This product is only short-lived and undergoes a rearrangement into isobarbituric acid [reaction (124)] and hydration yielding Ug [reaction (123)]. Such reactions which will be discussed in more detail below.

Table 10.15. γ-Radiolysis of N_2O/O_2-saturated solutions of Ura (2×10^{-4} mol dm^{-3}). Products and their *G* values (unit: 10^{-7} mol J^{-1}; Schuchmann and von Sonntag 1983)

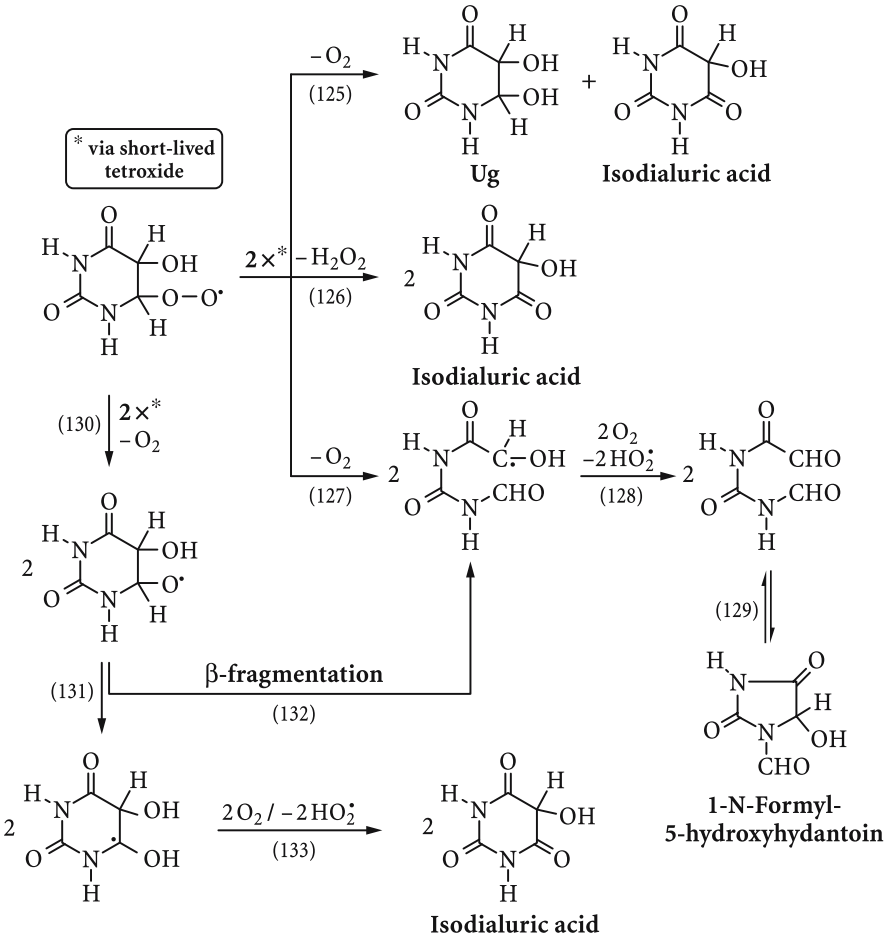
Product	pH 3.0	pH 6.5	pH 10.0
5,6-*cis*-Dihydroxy-5,6-dihydrouracil (Ug)	0.6	0.9_5	1.4_5
5,6-*trans*-Dihydroxy-5,6-dihydrouracil (Ug)	0.5	1.1	1.0
Isobarbituric acid	0	0.2	1.2_5
1-*N*-Formyl-5-hydroxyhydantoin	1.6_5	1.4_5	0.2
Dialuric acid	0.9_5	0.4	0.2
Isodialuric acid	0.1	0.2	0.1
5-Hydroxyhydantoin	0.4	0.4	0.3
Unidentified products	0.9_5	0.6	0.9_5
Hydrogen peroxide	n.d.	3.0	n.d.
O_2 consumption	n.d.	5.0	n.d.
Ura consumption	5.1	5.5	5.4

n.d., Not determined



In acid solutions, but also in neutral solutions at a high steady-state radical concentration, $O_2^{\bullet -}$-elimination becomes too slow to be of importance as compared to the bimolecular decay of the peroxyl radicals. This leads to a very different product distribution (Table 10.15).

Typical decay pathways, with tetroxides as short-lived intermediates (Chapter 8.8), lead to Ug, dialuric acid and O_2 [reaction (125)], as well as to two mol dialuric acid and H_2O_2 [reaction (126)].



Elimination of O_2 with concomitant fragmentation yields 1-*N*-formyl-5-hydroxyhydantoin [reactions (127-129]. The peroxyl radicals also give rise to oxyl radicals upon their bimolecular decay [reaction (130)], an alternative route to 1-*N*-formyl-5-hydroxyhydantoin [reactions (132), (128) and (129)]. In competition, these oxyl radicals may also undergo a 1,2-H-shift [reaction (131)], and after addition of oxygen eliminate $HO_2^{\bullet}/O_2^{\bullet -}$ [reaction (133)]. Thus also under such conditions, $O_2^{\bullet -}$ are likely intermediates. They are expected to react with other peroxyl radicals present to yield the corresponding hydroperoxides. Such hydroperoxides are abundant intermediates in the radiolysis of air-saturated pyrimidine solutions (Cadet and Teoule 1975; Wagner et al. 1987, 1990a).

A detailed kinetic study is still missing for Cyt and its derivatives, but for dCyd product data are available (Wagner et al. 1999). They are compiled in Table 10.16 together with the products observed upon menadione photosensitization.

Table 10.16. Product yields (%) in the γ-radiolysis and menadione-sensitized oxidation of aerated aqueous solutions of dCyd (Wagner et al. 1999)

Product	γ-Radiolysis	Photosensitization
5,6-Dihydroxy-5,6-dihydro-2′-deoxyuridine (Ug)	28	27
5-Hydoxy-2′-deoxycytidine	12	7
N_1-(dR)-Hydroxyhydantoine	3	2
N_1-(dR)-Formamide (Fo)	12	2
N_1-(dR)-1-Carbamoyl-3,4-dihydroxy-2-oxoimidazolidine	17	6
Aminocarbonyl[2-dR)-amino]-2-oxomethylcarbamic acid	3	2
N_1-(dR)-Biuret	3	2
5′,6-Cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine	2	Not detected
dUrd	Not detected	36
Cyt	12	4
2-Deoxyribono-1,4-lactone, 2-dRL	Traces	4

In the γ-radiolysis, similar reactions are expected to occur as discussed above for the Ura system. Here, however, about equal amounts of $^{\bullet}OH$ and $O_2^{\bullet -}$ are formed initially and thus the latter will play an even larger role on the way to the products, and thus it is even more difficult to come up with a well-substantiated reaction scheme. An interesting product is 5′,6-cyclo-5-hydroxy-5,6-dihydro-2′-deoxyuridine. Its formation is discussed below in the context of the reactions of alkyl radicals.

Photooxidation and γ-radiolysis agree in their products reasonably well as long as the base is damaged. The reason for this may be that in the photooxidation a dCyd radical cation / menadione radical anion pair is the first intermediate. The reaction of the dCyd radical cation with water could give rise to $^{\bullet}OH$-adducts (as in radiolysis). The most important product, however, is dUrd, that is, deaminated dCyd (Table 10.16) which is not, and cannot be, formed upon radiolysis. The mechanism of its formation is not discussed in the original paper, but it is not unlikely that it results from an unstable intermediate (adduct?) formed in the reaction of the dCyd radical cation / menadione radical anion pair in the solvent cage.

With d(GpC) and d(CpG) γ-irradiated in N_2O/O_2-saturated solutions, the corresponding Ug and 1-carbamoyl-2-oxo-4,5-dihyroxyimidazolidine deriva-

tives were detected (Paul et al. 1987b). Dinuclosides with intact dCyd and an 8-oxo-G moieties were also observed. With d(TpA) and d(ApT), the Fo derivatives are the prominent products, but products with an aldehyde group at the free 5′-end were also detected (Paul et al. 1987a).

With d(TpA) and d(GpC) the same products as observed to be formed by ionizing radiation were detected upon autoxidation induced by ascorbate plus phosphate buffer (low amounts of transition metal ions are omnipresent; Arakali et al. 1988).

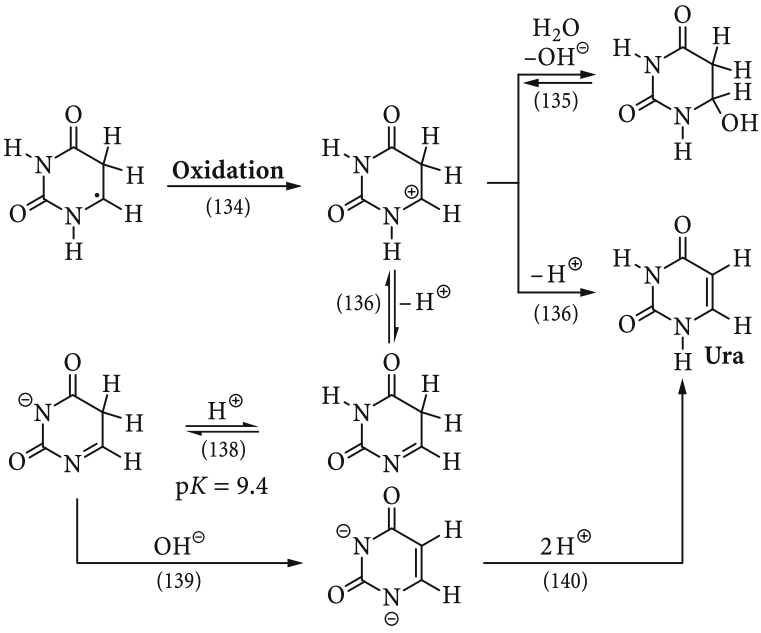
Purines. The reactions of the $^{\bullet}OH$-adduct radicals of the purines in the presence of O_2 are still very poorly understood. The dGuo *C*(8)-$^{\bullet}OH$-adduct is reported to react very fast with O_2 ($k = 4 \times 10^9$ dm^3 mol^{-1} s^{-1}; Candeias and Steenken 2000). There is no agreement as to the yield of 8-oxo-G under such conditions. In one report, its *G* value is given as 0.84×10^{-7} mol J^{-1} (Vieira et al. 1993), but a lower value of 0.3×10^{-7} mol J^{-1} has also been measured (von Sonntag and Schuchmann 2001). An even much lower value has been found in the γ-radiolysis of air-saturated dGuo solutions ($G = 0.0038 \times 10^{-7}$ mol J^{-1}; Svoboda and Harms-Ringdahl 1999). The latter group reports that in DNA *G*(8-oxo-G) is markedly higher (0.077×10^{-7} mol J^{-1}). Thus, considerably more work will be required not only to establish the yield of this crucial product, but also to shed some light on the mechanism of its formation in the presence of O_2. When Gua was oxidized with the Fenton reagent (0.15×10^{-3} mol dm^{-3} Fe^{2+} and 50×10^{-3} mol dm^{-3} H_2O_2) the 8-oxo-G yield "oscillated" over a time range of 20 min with very little 8-oxo-G to start with (White et al. 2003). This cannot be attributed to the Fenton reaction proper, since under these experimental conditions this reaction is completed after a few seconds (Chap. 2.5). Similar findings were reported for DNA. There is no mechanistic explanation yet for these intriguing observations.

10.3.6 Formation and Properties of Isopyrimidines

Isopyrimidines have been postulated (Haysom et al. 1972, 1975; Al-Yamoor et al. 1977; Asmus et al. 1978; Al-Sheikhly et al. 1984; Schuchmann et al. 1984a; Garner and Scholes 1985) or are likely to be involved (Holian and Garrison 1966; Teoule and Cadet 1974; Cadet 1980) as short-lived intermediates formed upon oxidation of pyrimidine-6-yl radicals carrying an H-atom at *N*(1) [reactions (134) and (137)]. Isopyrimidines are also intermediates in the photohydration reaction (Al-Yamoor et al. 1977; Garner and Scholes 1985). In the Ura system, for example, the carbocation (immonium ion) is the first intermediate which either deprotonates at *C*(5) yielding Ura [reaction (136)] or reacts with water giving rise to the hydrate [reaction (135)]. The latter reaction is reversible in acid solution. Mechanistically, reaction (–137) accounts for the well-known water elimination of the photohydrate (for its stabilty in near-to-neutral solutions see Carter and Greenberg 2001; the other isomer with the OH group at *C*(5) is not acid labile, and these two hydrates may be distinguished based on their different behavior in acid solution).

Table 10.17. Rate constants of isopyrimidine → pyrimidine rearrangements at 20 °C. (Schuchmann et al. 1984a)

Isopyrimidine	Neutral spont. s^{-1}	Neutral H^+-catal. dm^3 mol^{-1} s^{-1}	Neutral OH^--catal. dm^3 mol^{-1} s^{-1}	Anion spont. s^{-1}	Anion OH^--catal. dm^3 mol^{-1} s^{-1}
Isouracil	3000	1.8×10^7	Absent	50	4.9×10^5
3-Methyl-isouracil	2500	1.1×10^7	2.7×10^7	Absent	Absent
5-Hydroxy-isouracil	2000	2.6×10^7	Absent	< 50	$\times 10^5$



Deprotonation of the carbocation at *N*(1) [reaction (137)] is kinetically favored over the deprotonation at *C*(5) [reaction (136)]. Thus, isouracil is an important intermediate in these reactions. It is sufficiently long-lived to follow its reactions by pulse radiolysis. Isouracil is in equilibrium with its anion [equilibrium (138)]. This allows reactions (139) and (140) to proceed as well. However, when *N*(3) is substituted such as in 3MeUra, this additional pathway is no longer possible. For a compilation of rate constants see Table 10.17.

Table 10.18. Sites of H•-attack (in %) in Ura and some of its derivatives. (Das et al. 1985)

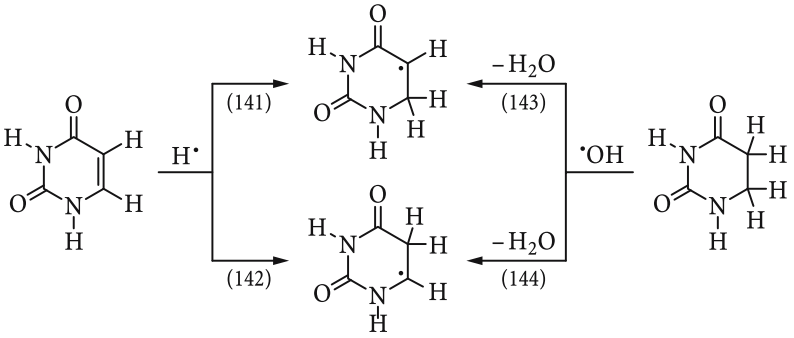
Substrate	*C*(5)	*C*(6)	Methyl group and sugar moiety
6MeUra	87	13	nil[a]
1,3Me$_2$Ura	71	29	nil[a]
Ura	69	31	–
poly(U)	60	40	nil[a]
Thy	37	59.5	3.5
Thd	32	62.5	5.5
1,3Me$_2$Thy	25	73	2.0

[a] Within experimental error

10.3.7 Reactions of the Hydrogen Atom

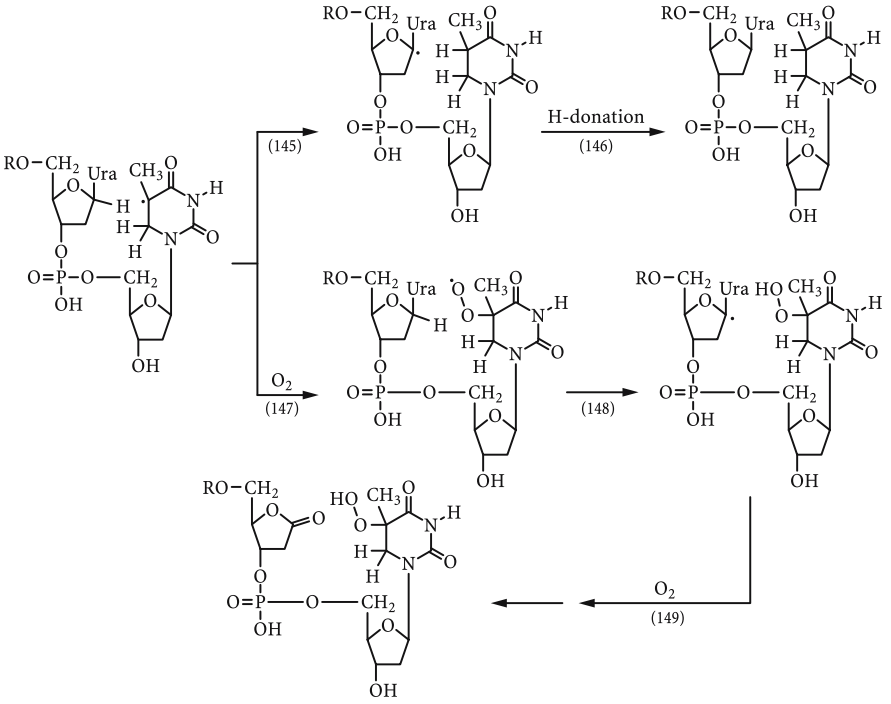
Similar to •OH, H• is an electrophilic radical (Chap. 4.4), and in its additions to C–C double bonds it has a strong preference for electron-rich sites (Das et al. 1985; Table 10.18). Thus, the *C*(5)-position is the preferred site of addition in the pyrimidine series as depicted here for Ura [reaction (141)].

The *C*(6)-yl radical is also formed upon the reaction of •OH with dihydropyrimidines [reaction (144); Schuchmann et al. 1984b], as can be seen from the data compiled in Table 10.19. For a quantum-mechanical study of their structure and EPR coupling constants see Jolibois et al. (1998).



Due to the much lower rate of H-abstraction by H• as compared to •OH, H-abstraction is generally of much lower if any importance (Table 10.18).

The 5,6-dihydrothymid-5-yl radical has been produced independently by photolyzing an adequately substituted derivative (Barvian and Greenberg 1995). This radical has a rather low H-abstracting power, and very good H-donors such as 1,4-dihydrocycloexadiene are required to afford substantial yields of H_2Thd beyond simple disproportionation. The latter has been shown to proceed both by H-transfer to *C*(5) as well as to *O*(4). In the case of the dinucleotide dUrdpThd, the 5,6-dihydrothymid-5-yl abstracts the *C*(1′)-H of the neighboring dUrd unit [reaction (145); Tallman and Greenberg 2001]. The rate constant for this intramolecular H-transfer has been estimated to range between 115 and 400 s^{-1}. In the presence of O_2, an interesting selective intramolecular *C*(1′)-H abstraction by the 5*S*-diastereoisomer of the *C*(5) peroxyl radicals takes place. This leads to a damage amplification (tandem lesion) with a 2′dRL and a 5OOH6HThy lesion [reactions (147)-(149)].

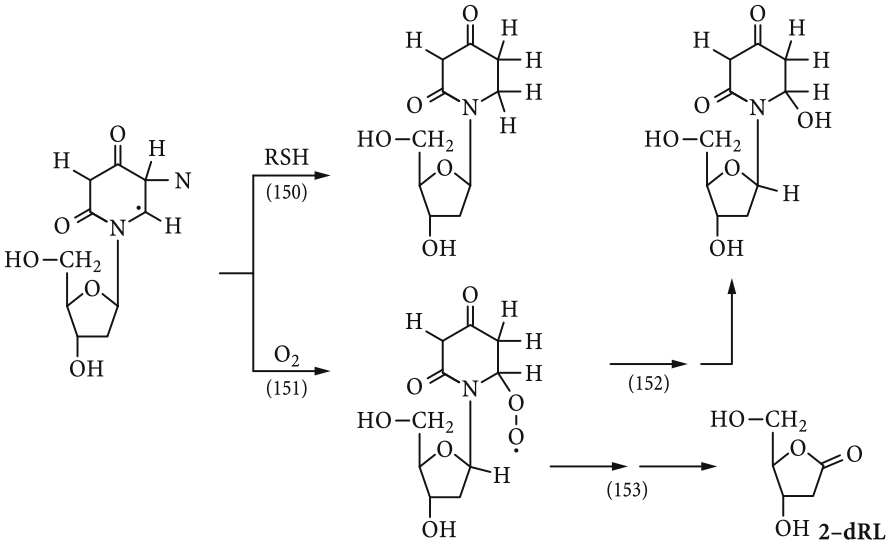


The 5,6-dihydro-2′-deoxyuridin-6-yl was generated specifically by photolyzing the respective tertiary butyl ketone (Carter and Greenberg 2003). It reacts fast with 2-mercaptoethanol [reaction (150); $k = 8.8 \times 10^6$ dm^3 mol^{-1} s^{-1}] and noticeably slower with 2,5-dimethyltetrahydrofurane (31 dm^3 mol^{-1} s^{-1}). In the presence of O_2, 6-hydroxy-5,6-dihydro-dUrd is the major product [reactions (151) and (152)]. Interestingly, 2-dRL is also formed under such conditions which is believed to be formed by an intramolecular *C*(1′)-H abstraction via a six-membered transition state [reaction (153); for this type of reaction see also Chap. 8.6].

Table 10.19. Pattern of •OH-radical attack (in %) on H_2Ura and some of its methyl derivatives. The •OH-balance is sometimes significantly below 100%, partly due to the inefficiency of the method for the detection of oxidizing *C*(5)-yl radicals. (Schuchmann et al. 1984b)

Substrate	*C*(5)	*C*(6)	Methyl	Total
H_2Ura	5	90	–	95
1MeH_2Ura	≈ 2	61	29	92
3MeH_2Ura	≈ 2	72	10	84
1,3Me$_2$H$_2$Ura	≈ 2	≈ 62	30[a]	94
H_2Thy	4	74	6	84
6MeH_2Ura	≈ 2	84	6	92

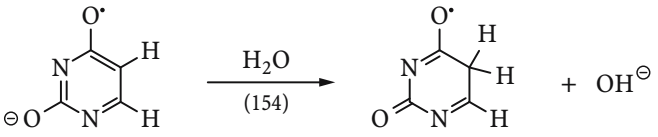
[a] ≈ 22% at *N*(1)CH_3 and 8% at *N*(3)CH_3



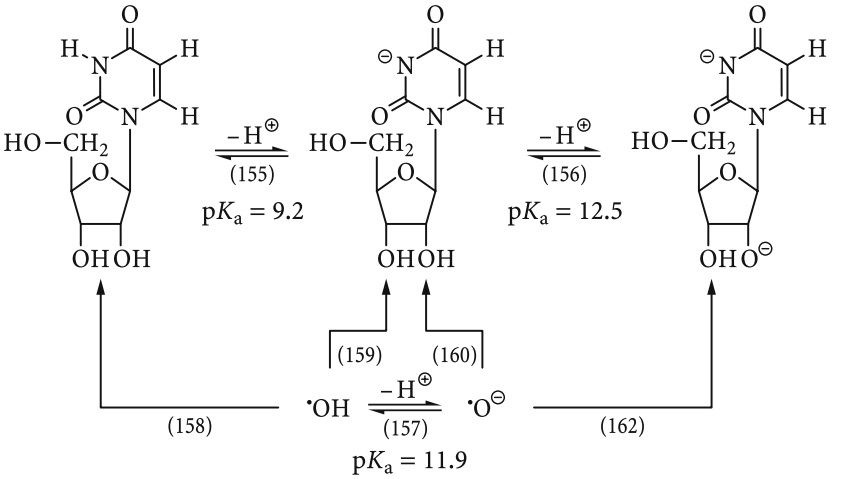
In DNA, the *C*(1′)-H is hidden in the minor groove and hence difficult to access by reactive free radicals such as •OH (Chap. 12.2). If the above type of reaction would occur within DNA, it would lead to a tandem lesion (for their biological importance see Chap. 12.5).

10.3.8 Reactions of $O^{\bullet-}$

At very high pH, $^{\bullet}OH$ deprotonates ($pK_a(^{\bullet}OH) = 11.9$), and the reactions that are observed are due to that of $O^{\bullet-}$. The reactions of $O^{\bullet-}$ are typically one order of magnitude lower than those of $^{\bullet}OH$. In some of the studies reported below, experiments have been carried out at pH 13. This pH is not high enough to exclude major contributions of $^{\bullet}OH$. This has to be taken into account when consulting these papers. In contrast to $^{\bullet}OH$, $O^{\bullet-}$ only very reluctantly adds to C-C double bonds, but it is a good H-abstractor and has also a considerable oxidation power (Chap. 3.1). At pH 13, all nucleobases are deprotonated, and their reduction potentials are quite low (ranging between 0.63 V (Gua) and 0.88 (Ura); Jovanovic and Simic 1986). For Ura, Cyt, Ade and Gua, it has been suggested that the reaction of $O^{\bullet-}$ with their anion and dianions occurs by ET, a reaction that is also given by $Br_2^{\bullet-}$ under these conditions (Ioele et al. 1998). In the case of Cyt, an H-abstraction from the amino group has also been considered as an alternative. With Thy, 1MeUra and 1MeCyt H-abstraction at methyl dominates (Ioele et al. 1998), and this is also reported for other methyl-substituted pyrimidines (Luke et al. 2003). The one-electron oxidized nucleobase (di)anions of Ura, Thy (formed via $Br_2^{\bullet-}$) and Ade decay unimolecularly with rate constants near $10^4\ s^{-1}$. Two alternatives are envisaged, nucleophilic substitution at *C*(6) by OH^- or protonation at carbon by water [reaction (154)]. Since the rate of reaction does not depend on the OH^- concentration, the second alternative may be favored. It is analogous to the also slow protonation at carbon of the radical anion that is discussed in the next paragraph.



In nucleosides, *G*(base release) increases dramatically at high pH (Fujita 1984). This has been suggested to be due to an OH^--induced transformation of a base $^{\bullet}OH$-adduct radical into a sugar-centered radical that releases the base. However, it has been subsequently shown that this effect is due to the involvement of the basic form of $^{\bullet}OH$, the $O^{\bullet-}$ radical [Scholes et al. 1992; reactions (160) and (162)]. The $O^{\bullet-}$ radical undergoes H-abstraction from the sugar moiety rather than addition to the base (for rate constants see Chatgilialoglu et al. 2005). Some of these sugar-derived radicals will release the base (see below). Reactions (155)-(162) show the various pK_a values that are involved in the Urd system given as an example. The deprotonation of ribonucleosides at the 2′-position at high pH has recently been substantiated (Velikyan et al. 2001).



10.4 Radical Anions and their Protonated Forms

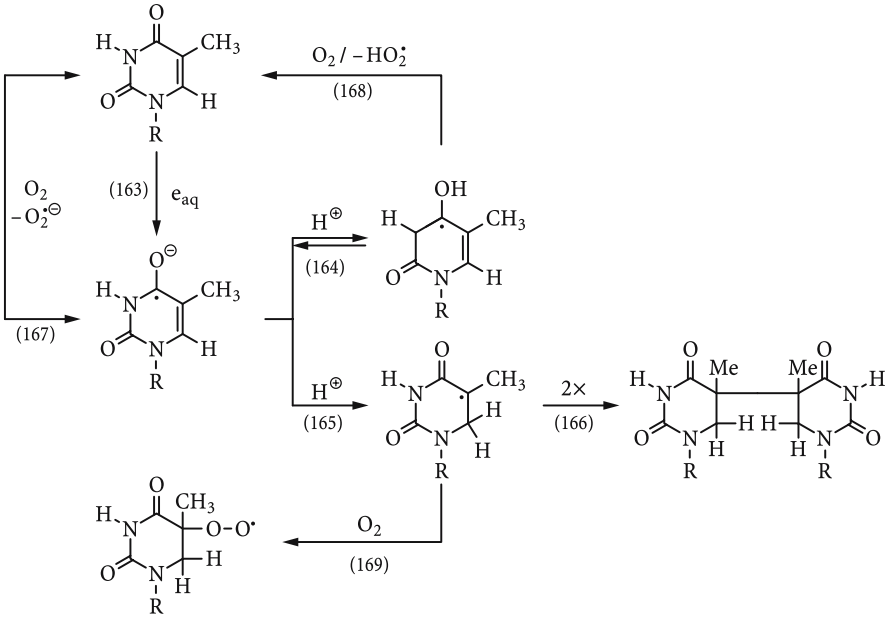
The radical anions may be formed by reacting the nucleobases with e_{aq}^- which may be either generated radiolytically or in a two-step reaction, e.g., in the laser flash photolysis of anthraquinonedisulfonate in the presence of pyrimidines (yielding the pyrimidine radical cation and an anthraquinonedisulfonate radical anion) and subsequent photoionization of the anthraquinonedisulfonate radical anion (Lü et al. 2001). The latter approach, combined with Fourier transform EPR spectroscopy, yielded detailed information as to the conformation of the radical anions of Ura and Thy in aqueous solution (for a discussion see Close 2002; Naumov and Beckert 2002). Similarly valuable EPR information has been obtained from γ-irradiated single crystals (cf. Box and Budzinski 1975; Box et al. 1975; Sagstuen et al. 1998).

Pyrimidines. The pyrimidines react with e_{aq}^- at practically diffusion-controlled rates (Table 10.6). The ensuing reactions of the radical anions of Ura and Thy are very well understood, while with that of Cyt some open questions still remain.

Thy and Ura behave like typical carbonyl compounds, and the first intermediate is a radical anion [reaction (163), in the case of Thy/Thd] which is in equilibrium with its *O*-protonated conjugate acid [equlibrium (164)].

Table 10.20. Rate constants for protonation of the pyrimidine radical anion at *C*(6) by $H_2PO_4^-$ using various approaches of evaluating the data (Deeble et al. 1985)

Pyrimidine	pK_a	$k/10^7$ dm^3 mol^{-1} s^{-1}
1,3Me$_2$Thy	7.2	1.6/0.74 /0.95
Thy	7.2	0.32/0.33
Thd	7.2	0.19/0.21
1MeUra	7.0	0.14/0.25
Ura	7.3	~0.1
Urd	7.2	~0.05/~0.05
6MeUra	7.3	<0.01



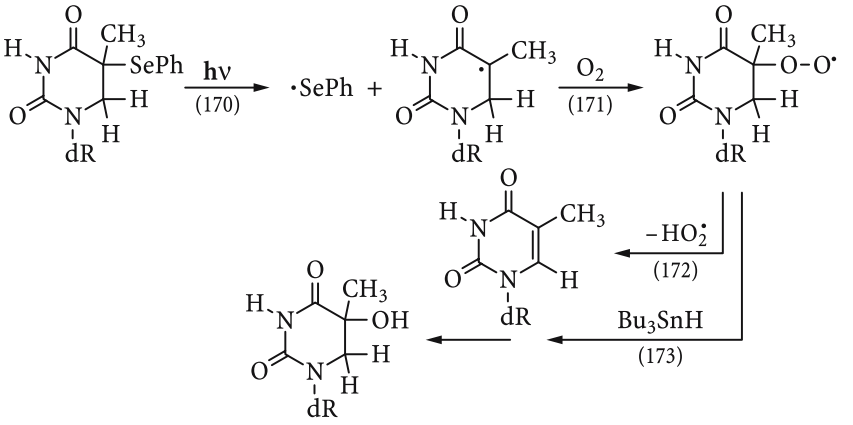
The other functional groups, especially the second carbonyl function, withdraws considerable electron density, and hence the pK_a values of these *O*-protonated radical anions are much lower than those of the simple α-hydroxyalkyl radicals (Thy: pK_a = 6.9 (Hayon 1969); Ura: pK_a = 7.2 (Shragge and Hunt 1974); the value of 4.3 reported for the pK_a for the protonated 5,6Me$_2$Ura radical anion (Aravindakumar et al. 1998) is surprising low and difficult to explain on the basis of the effects of methyl substituents on the pK_a values of α-hydroxyalkyl radi-

cals; Chap. 6.2). In contrast to a protonation at the heteroatom [reaction (164)], protonation at carbon [reaction (165)] is connected with some conformational changes, and thus the conversion into this thermodynamically more stable (for DFT calculations see Naumov and von Sonntag, unpublished results) isomer can only be observed on the pulse radiolysis time scale, when buffer is added to speed up the protonation/deprotonation reactions (Das et al. 1984; Deeble et al. 1985; for rate constants see Table 10.20; for an EPR study see Novais and Steenken 1986).

Together with this transformation, the redox properties of the radicals change dramatically. While the radical anion and its *O*-protonated conjugate acid are good reducing agents has the *C*(6) protonated tautomer oxidizing properties. This is also reflected in their reactions with O_2. The former two give rapidly rise to $HO_2^{\bullet}/O_2^{\bullet-}$ thereby restoring the pyrimidine [reactions (167) and (168); Deeble and von Sonntag 1987]. On the other hand, when O_2 reacts with the *C*(6) protonated tautomer [reaction (169)], the pyrimidine chromophore is destroyed in the subsequent reactions of the ensuing peroxyl radical. In the absence of O_2, the 5,5′-dihydrodimer is formed [reaction (166); Ito et al. 2002].

The radical anion can also be repaired by polyphenols such as rutin or quercitin (Shi et al. 2000b; Zhao et al. 2003). Yet, this repair is not related to their phenolic functions but is rather due to an ET to their carbonyl (flavone) functions.

Photolysis of 5,6-dihydro-5-selenophenyl-dTyd (Tallman et al. 1998) also affords the *C*(6)H$^{\bullet}$-adduct, and in the presence of O_2 the corresponding peroxyl radical [reactions (170) and (171)]. The latter may undergo $HO_2^{\bullet}$-elimination giving rise to Thd [reaction (172)] or, in the presence of tributyltinhydride, yields 5,6-dihydro-5-hydroxy-dTyd [reaction (173)]. The ratio of these two products depends on the tributyltinhydride concentration, and from such data the ratio of the rate constants of reactions (172) and (173) has been calculated at 1.3×10^{-2} mol dm^{-3}.

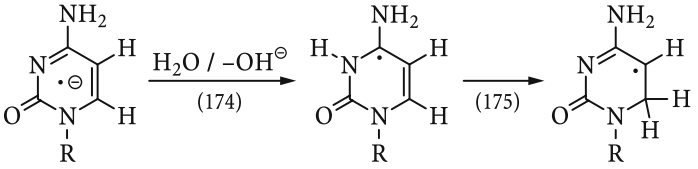


The rate of reaction (173) has been estimated at ~65 s^{-1} assuming a reactivity of tributyltinhydride similar to that of a thiol. For the latter a rate constant was taken from the work of Schulte-Frohlinde et al. (1986) that later had to be revised to a much lower value (Hildenbrand and Schulte-Frohlinde 1997; Lal et al. 1997).

Thus the rate of $HO_2^{\bullet}$-elimination must be considerably slower (nearer to 1 s^{-1}), but still fast enough to play a role in DNA free-radical chemistry. Reaction (172) is reminescent of the $HO_2^{\bullet}$-elimination from β-hydroxycyclohexadienylperoxyl radicals (derived from the reaction of $^{\bullet}OH$ with benzene in the presence of O_2; Chap. 8.4). In this system, the reaction is much faster (k = 800 s^{-1}; Pan et al. 1993b); possibly due to the gain in energy in the course of the re-aromatization].

In this context it is worth considering that in the free-radical chemistry of DNA the *C*(6)-$^{\bullet}$OH-adducts radicals are certainly of a greater importance than the *C*(6)-H-adduct radicals investigated here. If the benzene system is a good guide $HO_2^{\bullet}$-elimination from hydroxycyclohexadienylperoxyl radicals is noticeably slower than that from cyclohexadienylperoxyl radicals (Pan et al. 1993a), i.e., the rate of $HO_2^{\bullet}$-elimination from 5,6-dihydro-6-hydroxy-thymidine-5-peroxyl radicals may even be slower.

The Cyt electron adduct is rapidly protonated by water [reaction (174); $t_{1/2}$ < 200 ns, Hissung and von Sonntag 1979; $t_{1/2}$ < 20 ns, Visscher et al. 1988] most likely at *N*(3) at or at the amino group (Symons 1990; Hüttermann et al. 1991; Podmore et al. 1991; Barnes and Bernhard 1994).



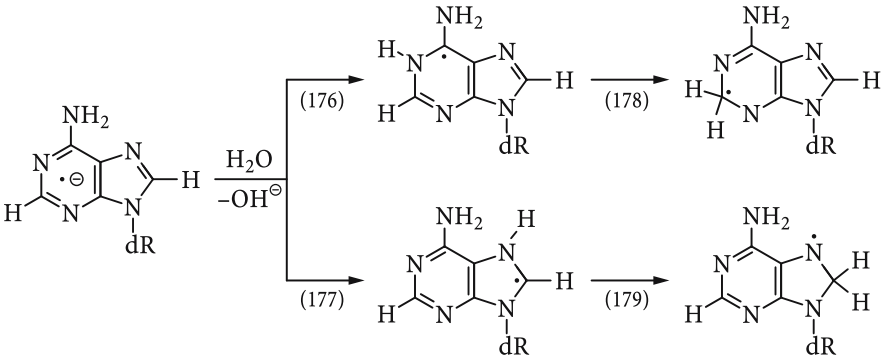
The resulting neutral radical must have a pK_a value ≥ 11, as shown by conductance measurements in pulse radiolysis (Hissung and von Sonntag 1979). The absence of any noticeable changes in the absorption spectrum of the radical derived from Cyd in the pH range 6-13 suggests that its pK_a value is even >13 (Steenken et al. 1992). Like the situation in the corresponding Thy system, the heteroatom-protonated species is not thermodynamically favored and subsequent (irreversible) protonation seems to occur at carbon [reaction (175)], albeit with a rate constant (estimated at 2.5 × 10^3 s^{-1}; Nese et al. 1992) too slow to be measured by pulse radiolysis.

For studies on the formation of the Ura-H$^{\bullet}$-adducts at *O*(4) and *C*(5) in the gas phase see Syrstad et al. (2001) and Wolken and Turecek (2001).

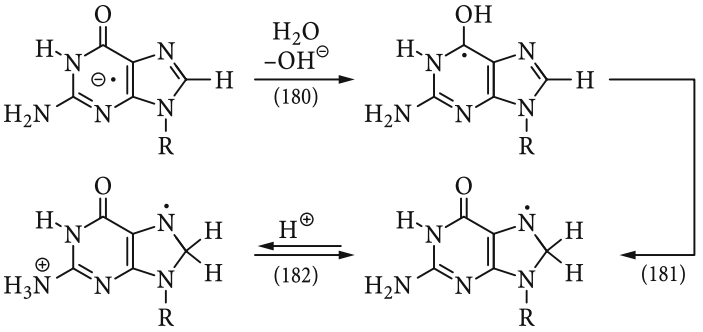
Purines. With the purines, H$^{\bullet}$ and e_{aq}^- react at close to diffusion-controlled rates. Besides that, very little is known about the reactions of H$^{\bullet}$. However, H$^{\bullet}$ and $^{\bullet}$OH being both electrophilic radicals (Chaps 3.2 and 4.4), the position-specificity for the addition of H$^{\bullet}$ should be very similar to that of $^{\bullet}$OH.

The properties of the purine radical anions formed by their reaction with e_{aq}^- resemble those of the Cyt radical anion. Because the pK_a values of the heteroatom-protonated conjugate acid of the purine radical anions are very high, the purine radical anion are rapidly protonated by water (Hissung et al. 1981a; Visscher et al. 1987; von Sonntag 1991; Candeias and Steenken 1992a; Candeias et al. 1992; Aravindakumar et al. 1994). For example, the dAdo radical anion

is protonated by water with a rate of ≥1.4 × 10^8 s^{-1} (Visscher et al. 1987). Such a rapid protonation reaction occurs typically at a heteroatom [reactions (176) and (177); note that protonation can also occur at other nitrogen atoms]. The radical anion and also their heteroatom-protonated conjugate acids have reducing properties. Thus, the latter react readily with oxidants such as *p*-nitroacetophenone [k = 5 × 10^9 dm^3 mol^{-1} s^{-1}; Hissung et al. 1981a) and methylviologen [k = 2.5 × 10^9 dm^3 mol^{-1} s^{-1}; Candeias and Steenken 1992a]. Interestingly, the oxidation by *p*-nitroacetophenone does not fully restore dAdo, but a product is formed which has a pK_a value of 8.8, and it has been suggested that this product might contain the elements of water. Alternatively, the carbocation at *N*(6) formed upon oxidation may deprotonate at *N*(6) yielding the imino analog of 2'-deoxyinosine [pK_a(inosine = 8.8)] in competition to a deprotonation at *N*(1) that restores dAdo. The considerable change at high pH in the recorded UV absorption spectra which occurs at approximately pH 10.5 (Moorthy and Hayon 1975) is not due to a deprotonation reaction, but a rearrangement of heteroatom protonated radicals into carbon-protonated ones [reactions (178) and/or (179); Hissung et al. 1981a].

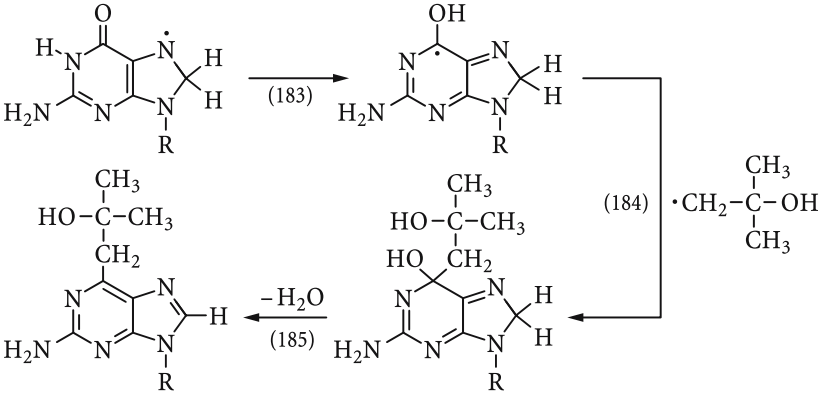


In Guo, after the very fast protonation of the electron adduct by water at the heteroatom [$k \geq 10^7$ s^{-1}, von Sonntag 1991; Candeias et al. 1992; at *O*(6), *N*(3) or *N*(7), cf. reaction (180)], a rapid transformation occurs [reaction (181); k(in H_2O) = 1.2 × 10^6 s^{-1}, k(in D_2O) = 1.5 × 10^5 s^{-1}] which is also catalyzed by phosphate buffer (k = 5.9 × 10^7 dm^3 mol^{-1} s^{-1}) which has been attributed to a protonation at *C*(8) (Candeias et al. 1992). This assignment is based upon solid-state EPR data, where *C*(8)-H•-adduct is the thermodynamically most stable H•-adduct radical (Rakvin et al. 1987; for DFT calculations see Naumov and von Sonntag, unpubl. results). The high solvent kinetic isotope effect of k_H/k_D = 8 is a strong indication that a proton is transferred in the rate-determining step. The magnitude of the rate of phosphate buffer catalysis points to a protonation at carbon (for a similar reaction observed with the Thy radical anion see Table 10.20). The *C*(8)-H•-adduct has a pK_a value of 5.4 [equilibrium (182)].



The *C*(8)-H$^{\bullet}$-adduct is only a weak oxidant. It does not react with methylviologen (E = -0.44 V/NHE; $k \leq 10^7$ dm^3 mol^{-1} s^{-1}), but it is quite readily oxidized by $Fe(CN)_6^{3-}$ (E = 0.35 V/NHE; $k = 5.6 \times 10^8$ dm^3 mol^{-1} s^{-1}).

When dGuo radical anion and the *t*BuOH-derived radical are generated side by side, a highly fluorescent product is formed [reactions (183)–(185); Yamamoto et al. 1995; Mandal and Yamamoto 1986].

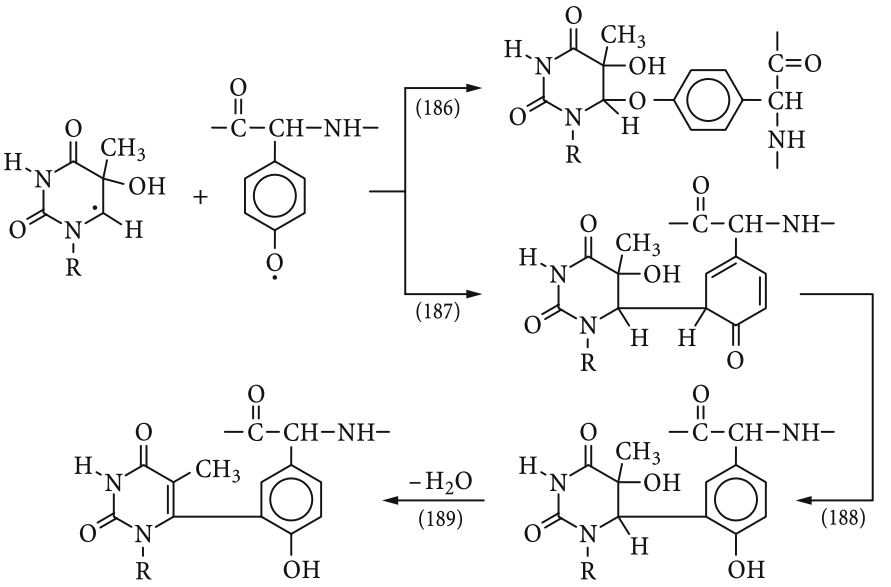


The mechanism presented here is somewhat at variance with that proposed by the authors (Yamamoto et al. 1995) who suggested that the *t*BuOH-derived radical adds to the primarily formed electron-adduct radical. Since this has been shown above to have only a very short lifetime, it will not be capable of undergoing bimolecular recombination reactions. An isomerization of *C*(8)-H$^{\bullet}$-adduct [reaction (183)] followed by an addition of the *tert*-butanol-derived radical and water elimination [reactions (184) and (185)] is not in conflict with the above pulse radiolysis results [note that the tautomerization reaction (183) cannot be excluded on the basis of the pulse radiolysis data].

10.5 Reactions with Alkyl and Thiyl Radicals

The question as to the reactions of alkyl radicals with nucleobases is a very important one. For example, α-hydroxyalkyl radicals, generated in the reaction of reactive free-radicals with alcohols, are capable of inactivating biologically active DNA (Nabben et al. 1983). Moreover, alkyl-type radicals are formed when reactive radicals such as •OH and H• add to the nucleobases or abstract an H atom from the sugar moiety of DNA. Thus, in principle, these DNA radicals thus generated could add to a neighboring nucleobase if the rate of reaction is sufficiently high and steric conditions allow the reaction to proceed. In DNA, this kind of reaction may lead to an intramolecular cross-link, e.g. by forming a small loop. Furthermore, DNA histone cross-linking may occur if a protein-derived radical would add to one of the DNA bases. Mechanistically, one has to clearly distinguish such addition reactions from the trivial case, where two base radicals or a base radical and a protein radical recombine. This trivial case is well documented (Yamamoto 1973; Grachev et al. 1983, 1986; Dizdaroglu and Simic 1984a,b; Dizdaroglu and Simic 1985a,b; Simic and Dizdaroglu 1985; Karam et al. 1986; Gajewski et al. 1988; Margolis et al. 1988; Dizdaroglu and Gajewski 1989; Dizdaroglu et al. 1989; Gajewski and Dizdaroglu 1990; Gajewski et al. 1990).

A case in point is the combination of a Thy •OH-adduct with a tyrosine-derived phenoxyl radical [reaction (186); Simic and Dizdaroglu 1985].



Apparently, the phenoxyl radical reacts also at carbon [reaction (187); the typical site for self-termination; Jin et al. 1993, 1995]. The resulting product undergoes an H-shift and eliminates water [reactions (188) and (189)]. The first step is certainly very fast and is expected to occur on the sub-ms time scale (cf. Capponi

et al. 1986). The second step will be much slower and may only occur during the work-up (cf. the slow – and proton-catalyzed – water elimination of bis(hydroxy cyclohexadienyl) into biphenyl; Mark et al. 2003).

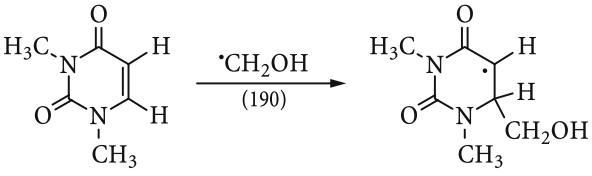
These reactions are only trivial as far as their chemistry is concerned (recombination of radicals and subsequent water elimination). This does not mean, however, that these reactions are of little importance in cellular-DNA free-radical chemistry.

It will be shown below that alkyl radicals add predominantly at the *C*(6)-positions of the pyrimidines and, when products as shown above are found after •OH-attack in very complex systems such as nucleohistones (e.g., Gajewski et al. 1988; Dizdaroglu and Gajewski 1989; Dizdaroglu et al. 1989; Gajewski and Dizdaroglu 1990) or Thy dimers in polydeoxythymidylic acid (Karam et al. 1986), it cannot be fully excluded that they are formed via the trivial two-radical recombination mechanism.

One of the first •OH-induced purine damage detected was in the 5′,8-cyclonucleotides. This lesion was later also observed in DNA (Chap. 12.5). In the following, the non-trivial case, the reactions of organic radicals with pyrimidines and purines will be discussed, and a special section will devoted to 5′,8-cyclonucleosides and nucleotides whose mechanism of formation has been found to be very complex.

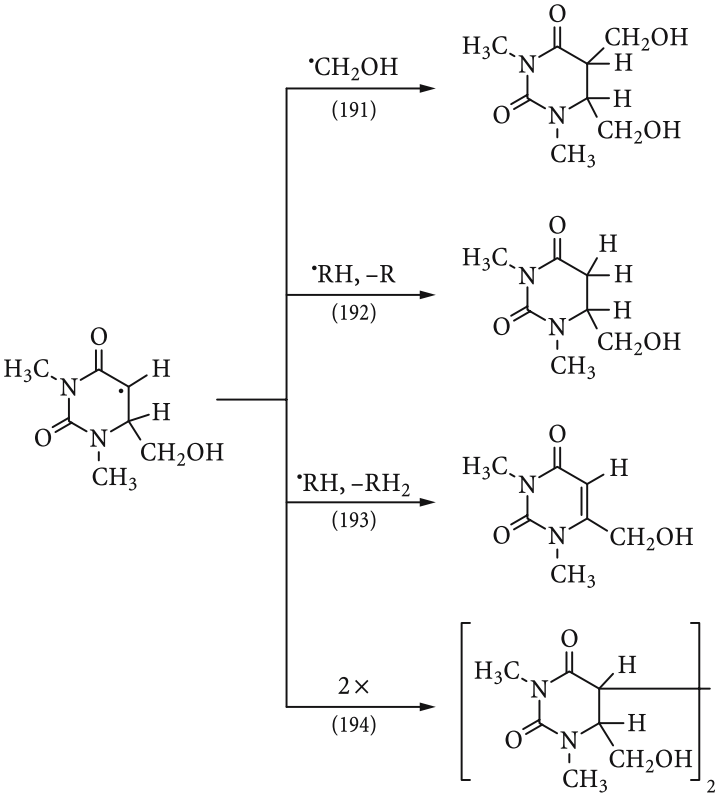
Pyrimidines. Alkyl radicals are nucleophilic radicals and add to the *C*(6)-position of pyrimidines rather than to the *C*(5)-position as the electrophilic •OH and H• do. The reactions of pyrimidines with α-hydroxyalkyl radicals that can be readily generated photolytically or radiolytically have been investigated intensively (Brown et al. 1966; Zarebska and Shugar 1972; Leonov et al. 1973; Frimer et al. 1976; Shetlar 1976, 1979, 1980; Ekpenyong and Shetlar 1979; Cadet et al. 1981; Ishida et al. 1985, 1986; Schuchmann et al. 1986). In the earlier radiolytic studies (Brown et al. 1966; Zarebska and Shugar 1972; Cadet et al. 1981), e_{aq}^- had been scavenged by the pyrimidines, and thus the pyrimidine electron adducts took part in the reactions. This has been avoided in a later study on the reactions of $^{\bullet}CH_2OH$ with $1,3Me_2Ura$ and $1,3Me_2Thy$ (Schuchmann et al. 1986), and the subsequent discussion is based on these results.

In the case of $1,3Me_2Ura$, all products (Table 10.21) can indeed be accounted for if in the primary step $^{\bullet}CH_2OH$ adds to the *C*(6) position [reaction (190)].



There is a considerable effect of the *G* values on the dose rate, and from this dose rate dependence it has been calculated that the rate constant of the addition of $^{\bullet}CH_2OH$ to $1,3Me_2Ura$ must be about 1×10^4 dm^3 mol^{-1} s^{-1}. Thus, the rate of this reaction is five orders of magnitude lower than that of •OH. Even at

the lowest dose rate used, not all $^{\bullet}CH_2OH$ were scavenged by the pyrimidine but also reacted with one another and with the 1,3Me$_2$Ura $^{\bullet}CH_2OH$-adduct [reactions (191)-(194)].



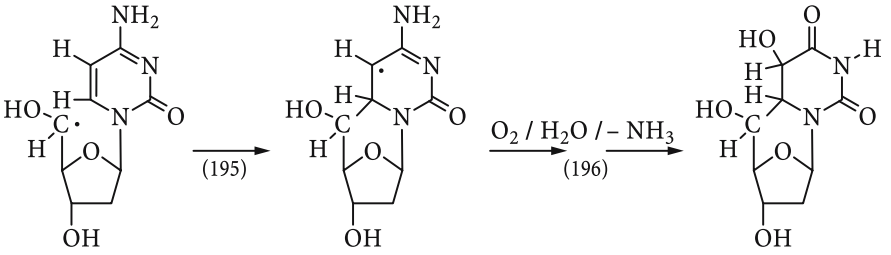
In this context, it may be of interest that hydroxymethylation of 1,3Me$_2$Ura (and its derivatives) by the Eu(III)/Eu(II) photoredox system in MeOH affords 5,6-dihydro-1,3-dimethyl-6-hydroxymethyluracil in close to 100% yield (Ishida et al. 1985). Apparently, the one-electron-reduced 1,3Me$_2$Ura, the *O*(4)-protonated 1,3Me$_2$Ura-4-yl radical, does not undergo sufficiently rapid isomerization into the thermodynamically more stable 1,3-dimethyl-5-hydro-uracil-5-yl radical under these conditions. Otherwise, 5,6-dihydro-1,3-dimethyl-5-hydroxymethyluracil would have been observed.

In dCyd, the α-hydroxyalkyl radical formed upon $^{\bullet}OH$ attack at the 5′-position must add to *C*(6) [reaction (195)] that fast that it is not scavenged effectively by O_2. An oxidation of the ensuing *C*(5) radical by O_2 and deamination are further likely steps [reaction (196)] to the observed product (Wagner et al. 1999; for its yield see Table 10.16).

Table 10.21. γ-Radiolysis if N_2O-saturated aqueous solutions of MeOH (0.5 mol dm^{-3}) in the presence of 1,3Me_2Ura (5×10^{-4} mol dm^{-3}). Products and their *G* values (unit: 10^{-7} mol J^{-1}) at different dose rates (unit: Gy s^{-1}; Schuchmann et al. 1986)

Product	0.18	0.026	0.0028
1,3-Dimethyl-6-hydroxymethyl-uracil	0.2	0.2	0.2
5,6-Dihydro-1,3-dimethyl-6-hydroxymethyluracil	0.85	1.7	2.1
5,6-Dihydro-5,6-di(hydroxymethyl)-1,3-dimethyluracil	0.95	0.7	0.6
5,5′-Bi-(5,6-dihydro-6-hydroxymethyl-1,3-dimethyl)uracilyl	0.45[a]	0.68[a]	0.95[a]
Formaldehyde	0.7	1.1	n.d.
Ethylene glycol	0.95	0.8	ND
1,3-Dimethyluracil consumption	2.4	3.2	4±1

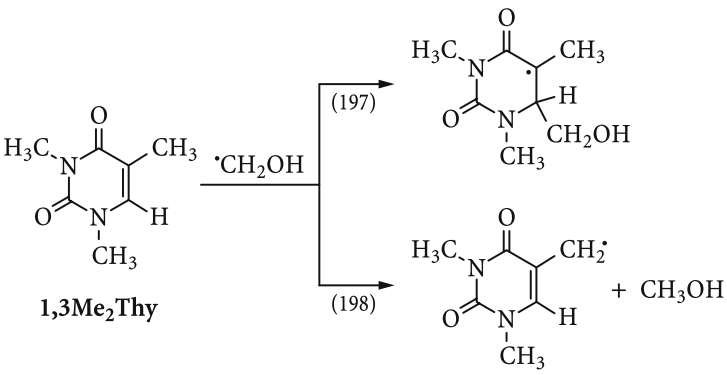
n.d., Not determined
[a] G values in monomer units



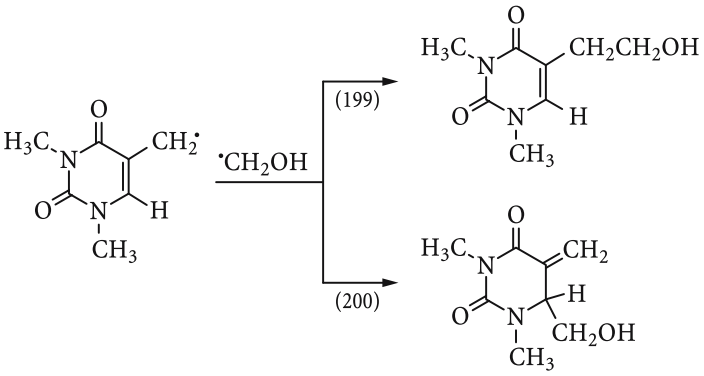
Although the O_2 concentration prevailing under the experimental conditions and other details such as products of competing reactions are not known exactly, one can estimate that the rate of the *C*(5′)→*C*(6) addition must be around 10^5 s^{-1}. This is the same order of magnitude as the addition of the *C*(5′) radical to the *C*(8) position in purines (see below).

The cyclonucleoside shown above has been synthesized and some of its properties such as piperidine stability were studied (Muller et al. 2002).

An interesting additional aspect offers the reaction of $^{\bullet}CH_2OH$ with 1,3Me_2Thy (Schuchmann et al. 1986). Here, not only is an addition to the *C*(6) position [reaction (197)] observed, but as much as 25% of $^{\bullet}CH_2OH$ abstract an H atom from the methyl group [reaction (198); see also Leonov et al. 1973; Livneh et al. 1982].



The allyl radical can react with another $^{\bullet}CH_2OH$ to form either the hydroxyethyl derivative [reaction (199)] or its isomer with an exocyclic double bond [reactions (200)].



The latter appears to be much more reactive towards $^{\bullet}CH_2OH$ than 1,3Me$_2$Thy itself, and reaches only such a low steady-state. Instead, its addition products (compiled in Table 10.22) are found to increase linearly with dose [for the first steps to these products see reactions (201)–(203)].

Table 10.22. γ-Radiolysis if N_2O-saturated aqueous solutions of MeOH (0.5 mol dm^{-3}) in the presence of 1,3Me_2Thy (5×10^{-4} mol dm^{-3}). Products and their *G* values (unit: 10^{-7} mol J^{-1}) at a dose rate of 0.19 Gy s^{-1}. (Schuchmann et al. 1986)

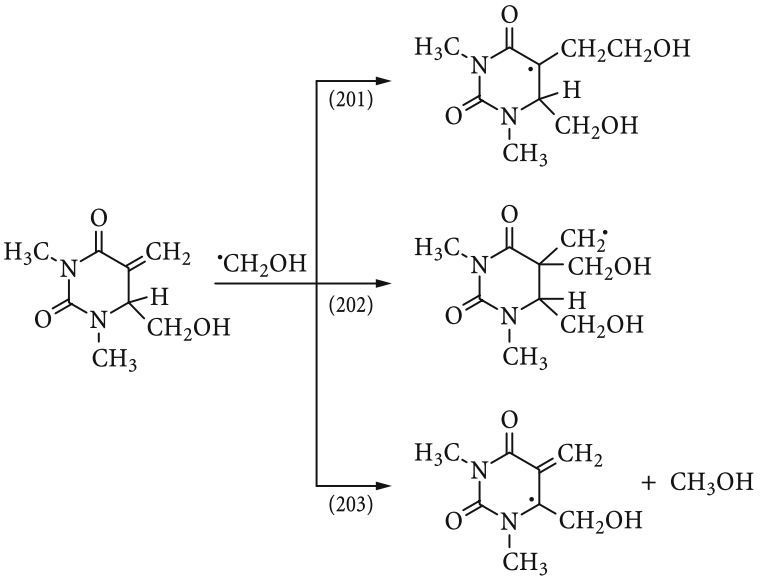
Product	*G* value
5,6-Dihydro-1,3-dimethyl-6-hydroxymethylthymine	1.6
5,6-Dihydro-5,6-di(hydroxymethyl)-1,3-dimethylthymine	0.3
1,3-Dimethyl-6-hydroxymethylthymine	0.08
1,3-Dimethyl-5-(2-hydroxyethyl)uracil	0.12
5,6-Dihydro-1,3-dimethyl-5-(2-hydroxymethyl)-6-hydroxymethyluracil	0.25
5-(2-Hydroxyethyl)-6-hydroxymethyluracil	0.03
5,6-Dihydro-5,6-di(hydroxymethyl)-1,3-dimethyl-5-(2-hydroxyethyl)uracil	0.09
5,6-Dihydro-5-(2-(1,3-dihydroxy)propyl)-1,3-dimethyl-6-hydroxymethyluracil	0.04
5,6-Dihydro-5-(5′,6′-dihydro-1′,3′,5′-trimethyluracil-6′-yl)-6-hydroxymethyl-1,3.5-trimethyluracil	0.01
5,5′-Bi-(5,6-dihydro-6-hydroxymethyl-1,3-dimethyl)thyminyl	0.12[a]
Formaldehyde	0.6
Ethylene glycol	n.d.
1,3-Dimethylthymine consumption	2.5

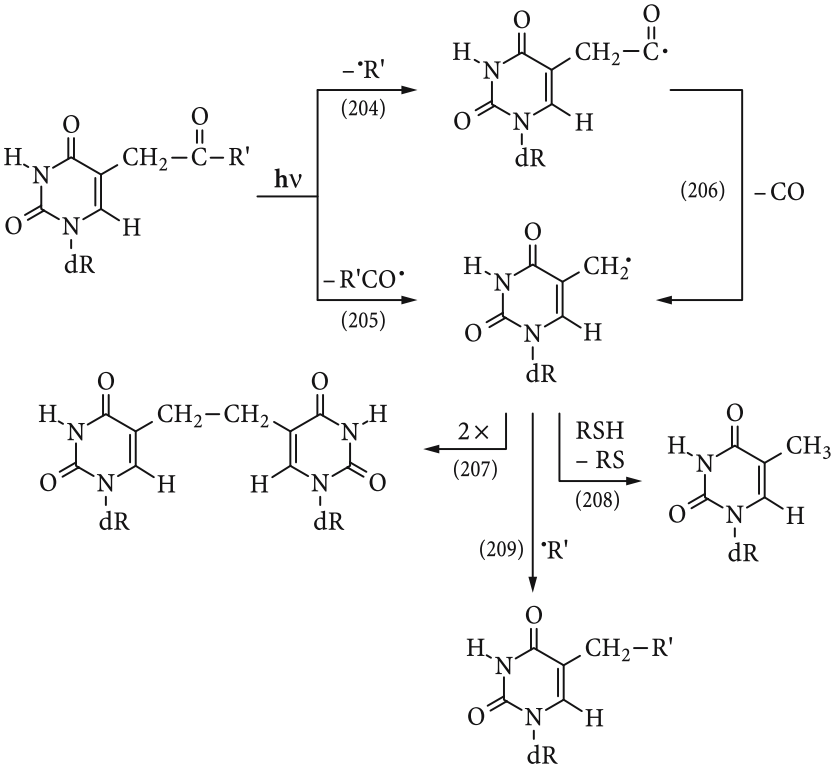
n.d., Not determined
[a] *G* value in monomer units

The allylic Thd radical has been generated photolytically [reactions (204–206); Anderson et al. 2000].

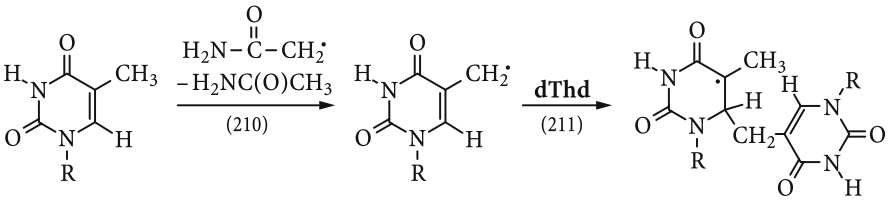
With R′ = benzyl and in the absence of O_2, the major product (73%) is the decarbonylation product [reaction (209); possible formed to a large extent within the solvent cage], and the dimer of the allylic radical [reaction (207)] is formed only in small amounts. Addition of a thiol increases the yield of Thd [reaction (208)]. If an evaluation of the data reported for the reduction of the allylic •OH-adduct to 1,3-cylohexadiene by a thiol (Pan et al. 1988), estimated at ~10^4 dm^3 mol^{-1} s^{-1}, is a good guide the rate constant for reaction (208) should be similar. This would revise an *assumed* rate constant of 10^6 dm^3 mol^{-1} s^{-1} and the conclusions as to the repairability of allylic Thy in DNA radicals by cellular thiols (Anderson et al. 2000).

The allylic radical has also by abstraction of the allylic hydrogen by the $H_2NC(O)CH_2$• radical in Thd [reaction (210] and its isomer has been produced from 6-chloromethyluracil by dissociative electron capture and also in low-tem-


(201)
(202)
(203)
+ CH3OH


hν
(204)
(205)
(206)
– CO
2×
(207)
RSH
– RS
(208)
(209)

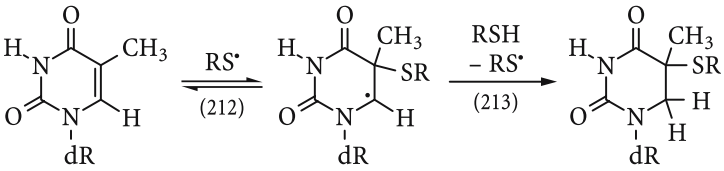
perature EPR experiments (Wang et al. 1997). Under such conditions the allylic radicals adds to the *C*(6)-position [reaction (211)], but there is no evidence for *C*(1′)-H abstraction. Thus, this observation may be of some importance regarding potential DNA-DNA cross-linking reactions.



In contrast, with dCMP as a substrate that lacks the weakly-bound allylic hydrogens, $H_2NCOCH_2^{\bullet}$ abstracts the *C*(1′)-H which is the most weakly-bound among the hydrogens of the sugar moiety (Wang et al. 1997).

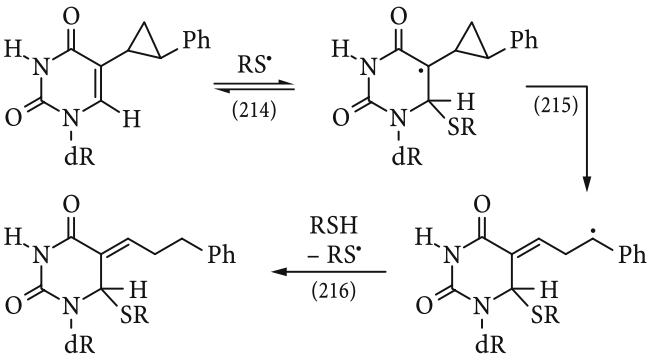
Addition of the (reducing) $CO_2^{\bullet-}$ radical to Thy (products not fully elucidated) shows that this radical behaves similar to other alkyl radicals, and reduction of Thy is certainly not its only reaction (Wada et al. 1982). Similar studies on Thd yielded the 5,5′-dihydrodimer ($G = 0.75 \times 10^{-7}$ mol J^{-1}) and H_2Thd ($G = 1.4 \times 10^{-7}$ mol J^{-1}; Nishimoto et al. 1983b). This does not yet fully account for the total $CO_2^{\bullet-}$ yield ($G \approx 6 \times 10^{-7}$ mol J^{-1}), but is a strong indication that reduction of Thd by $CO_2^{\bullet-}$ takes place in competition to addition.

The thiyl radical ($RS^{\bullet}$) is electrophilic and hence with pyrimidines its preferred site of attack is *C*(5) and to a lesser extent *C*(6) (Jellinek and Johns 1970; Varghese 1973, 1974; Shetlar and Hom 1987). Addition of $RS^{\bullet}$ to C-C double bonds is highly reversible [reaction (212), $k_{212} \approx 10^7$ dm^3 mol^{-1} s^{-1}, $k_{-212} \approx 10^5$ s^{-1} (Woijik et al. (2005); see also Chap. 7.4], and for fixing the $RS^{\bullet}$-adduct a fast subsequent reaction is required [reaction (213)].

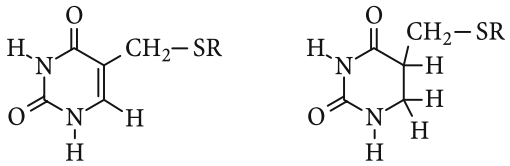


Considering that the equilibrium concentration of the *C*(6)-yl radical should be low, the major reaction should be a dimerization of $RS^{\bullet}$, but data confirming this are not available.

In reaction (214), the addition of $RS^{\bullet}$ to the *C*(6) position is followed by a very rapid and irreversible cyclopropyl carbinyl radical rearrangement [reaction (215); Carter et al. 2000]. The resulting radical is comparatively long-lived and can be reduced by the thiol [reaction (216)]. Thus in this example, the minor pathway is detected, while the other, being reversible, remains unobserved.



The acetone-sensitized photolysis of Thy in the presence of L-cysteine (R = cysteinyl), yields mainly 5-*S*-L-cysteinyl-5,6-dihydrothymine (see above), but also minor amounts of 5-*S*-L-cysteinylmethyluracil and 5-*S*-L-cysteinylmethyl-5,6-dihydrouracil are formed (Shetlar and Hom 1987; for an earlier study see Varghese 1973).



5-cytsteinyl-methyluracil

5-cytsteinyl-5,6-dihydro-methyluracil

The formation of 5-*S*-L-cysteinylmethyluracil may be taken as an indication that under these conditions photoexcited acetone also abstracts an H-atom from the methyl group of Thy or (less likely) that RS• is also capable of undergoing this reaction. The observation of 5-*S*-L-cysteinylmethyl-5,6-dihydrouracil is less easily explained. An exomethylene precursor as discussed above for the reaction of •CH_2OH with Thy could, in principle, account for it. Detailed mechanistic studies are, however, missing.

Purines. The reactions of purines with α-hydroxyalkyl and α-alkoxyalkyl radicals have been most intensively investigated (Elad et al. 1969; Steinmaus et al. 1969, 1971; Elad and Salomon 1971; Leonov et al. 1973; Salomon and Elad 1973; Leonov and Elad 1974a,b; Moorthy and Hayon 1975; Frimer et al. 1976; Aravindakumar et al. 1994), but the reactions of radicals derived from amino acids (Elad and Rosenthal 1969; Elad et al. 1969; Elad and Salomon 1971; Poupko et al. 1973; Salomon and Elad 1974) and amines (Elad and Salomon 1971; Salomon and Elad 1973) and of the methyl radical (Maeda et al. 1974) are also reported (for a review of the older work see Elad 1976).

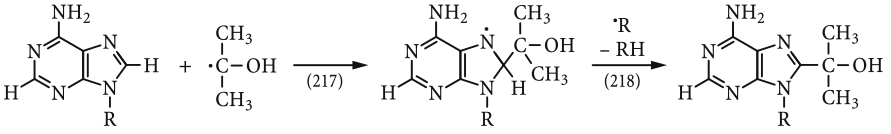
Alkyl radicals are nucleophilic radicals, and for this reason protonated purines react faster with alkyl radicals than the purines themselves (Table 10.22). Their rate also increases with increasing nucleophilicity of the radical, and hence •$C(CH_3)_2OH$ reacts much faster than •CH_2OH (Table 10.23). In fact, the

Table 10.23. Rate of reaction (unit: $dm^3\ mol^{-1}\ s^{-1}$) of α-hydroxyalkyl radicals with purines. (Aravindakumar et al. 1994)

Substrate	pH	$^{\bullet}C(CH_3)_2OH$	$^{\bullet}CH(CH_3)OH$	$^{\bullet}CH_2OH$
$HypH^+$	0.4	7.8×10^7	2.2×10^7	$\sim 5 \times 10^6$
Hyp	6.5	$\sim 4 \times 10^6$	n.d.	n.d.
$InoH^+$	0.4	3.2×10^7	3.0×10^7	$\sim 6 \times 10^6$
Ino	6.5	6.4×10^6	$\sim 5 \times 10^6$	n.d.
Ino anion	11.2	$\sim 10^6$	n.d.	n.d.
$AdoH_2^{2+}/AdeH^+$	0.4	1.2×10^8	4.3×10^7	4.4×10^6
$AdoH^+$	0.4	4.7×10^{7a}	1.5×10^7	$\sim 3 \times 10^6$
Ado	7	$< 10^{6a}$	n.d.	n.d.
Ado anion	13.6	$< 10^{6a}$	n.d.	n.d.
$dAdoH^+$	0.4	1.3×10^8	3.1×10^7	5.5×10^6
$GuoH^+$	0.4	4.3×10^7	1.8×10^7	5.7×10^6
$1MeGuoH^+$	0.5	8.0×10^{7a}	n.d.	n.d.
$dGuoH^+$	0.4	7.1×10^7	1.4×10^7	$\sim 4 \times 10^6$

n.d., Not determined
[a] From Moorthy and Hayon (1975)

reactivity of $^{\bullet}CH_2OH$ is so low, that its reaction is barely noticed, even at low steady-state concentrations of these radicals (Table 10.23). The product which is typically observed is the *C*(8)-alkylated purine, [e.g., reactions (217) and (218)].



As can be seen from Table 10.24, the consumption of the purines is noticeably higher than is accounted for by the *C*(8)-alkylated product. Thus, other, as yet not established, reactions must take place as well. It is recalled that the *C*(8) position rates among the sites preferred by electrophilic radicals and thus is possibly not the most preferred one for an attack by the nucleophilic alkyl radicals. Yet the EPR spectra of the intermediates formed in the reactions of the $H_2NCOCH_2^{\bullet}$ radical with Ade and Gua derivatives have also been interpreted as being mainly due to the *C*(8)-adduct radicals (Wang et al. 1997). Methyl radicals, generated in

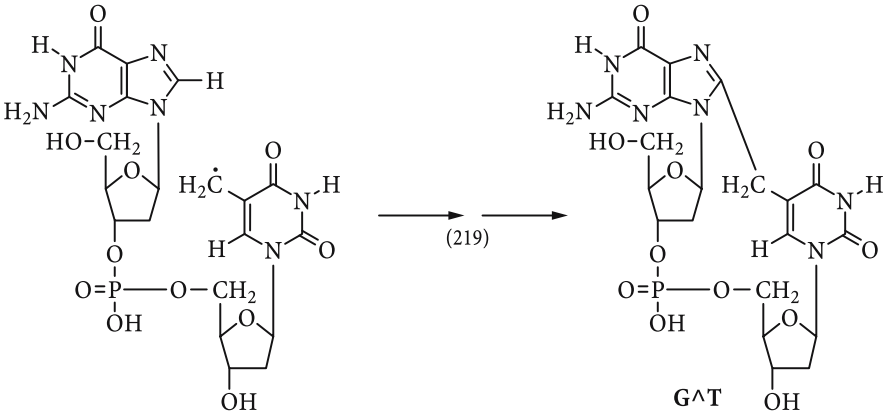
Table 10.24. Reactions of $^{\bullet}CH_2OH$ and $^{\bullet}C(CH_3)_2OH$ with Ade and Ino. Products and their *G* values (unit: 10^{-7} mol J^{-1}) determined under steady-state γ-radiolysis conditions (dose rate 0.28 Gy s^{-1}) at pH 6.5. (Aravindakumar et al. 1994)

Substrate	Radical	CH_2O	$(CH_2OH)_2$	$(CH_3)_2CO$	Ade consumption	Addition product
Ade	$^{\bullet}CH_2OH$	0.93	2.2	-	1.2	< 0.2[a]
Ade	$^{\bullet}C(CH_3)_2OH$	-	-	0.3	4.5	1.9
Ino	$^{\bullet}C(CH_3)_2OH$	-	-	3.8	-	n.d.

n.d., Not determined
[a] Structure not identified, assumed to be the *C*(8)-hydroxymethyl-substituted Ade

the photolysis of *N-tert*-butyoxy-2-pyridone in aqueous solution in the presence of dGuo, yielded 2.3% 8MedGuo and 0.27% 7MedGuo (Adam et al. 2002c). These yields are again very low, and the full product spectrum still awaits elucidation.

In Thy-Gua and Thy-Ade dinucleosides, the allylic Thy-derived radical gives rise to a tandem lesions denoted as G^T, T^G, A^T and T^A [e.g., reaction (219); Bellon et al. 2002].



In a GTG-containing 15-mer exposed to γ-irradiation in deaerated aqueous solutions, the G^T lesion strongly dominates over the T^G lesion, and in a ATA-containing 15-mer A^T dominates over T^A.

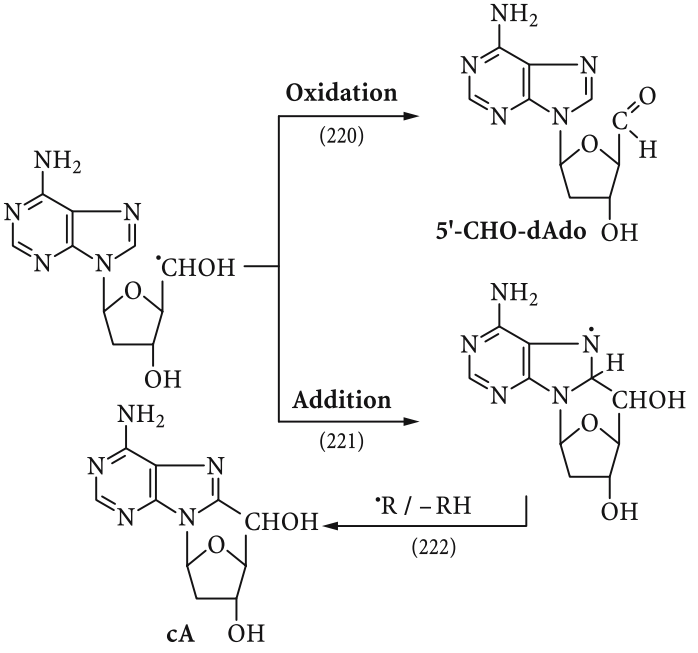
In the DNA of mammalian cells, Cyt at CpG sites is methylated (5MeCyt, mC). The radical at methyl in mCpG has been specifically generated upon photolysis of the corresponding SPh derivative. Cross-linking to C(8) of the neighboring Gua was found to be a major reaction both in d(mCpG) and d(GpmC) (Zhang and Wang 2003). Besides this cross-linking, an oxidation of MeCyt moi-

ety to $5CH_2OHCyt$ and 5CHOCyt in the absence of O_2 is observed but not yet understood.

An additional product, <d(TpG)>, that is formed in the benzophenone and menadione-sensitized oxidation of d(TpG), where the allylic Thy radical must have added to the *C*(4) position of the neighboring Gua moiety, is discussed below.

Purine-5′,8-cyclonucleosides and -cyclonucleotides. With purine nucleosides/nucleotides, cyclonucleosides/cyclonucleotides, cA and cG, have been observed as products formed upon •OH-attack (Hagen et al. 1965; Keck et al. 1966; Raleigh et al. 1976; Mariaggi et al. 1976; Raleigh and Blackburn 1978; Haromy et al. 1980; Berger and Cadet 1983a; Raleigh and Fuciarelli 1985; von Sonntag 1994). Reference material has been synthesized (Romieu et al. 1999a), and a method for their site-specific introduction into an ODN has been devised (Romieu et al. 1999c).

It is usually assumed that an H-abstraction at *C*(5′) by •OH (see, however, below) leads to the *C*(5′) radical which adds to *C*(8) [e.g., reaction (221); for details of this reaction see below, where experiments that lead to the specific generation of this radical are discussed]. In a disproportionation reaction with other radicals, the cyclonucleoside (cyclonucleotide) is formed [reaction (221)]. In competition, the *C*(5′) radical may be oxidized giving rise to the 5′-aldehyde [reaction (220)].



With AMP as substrate, *G*(cA) strongly depends on the pH. This has originally been explained by a variation of the rate constant of the H-abstraction reaction by •OH from the *C*(5′)-position as a function of the charge at the base and the

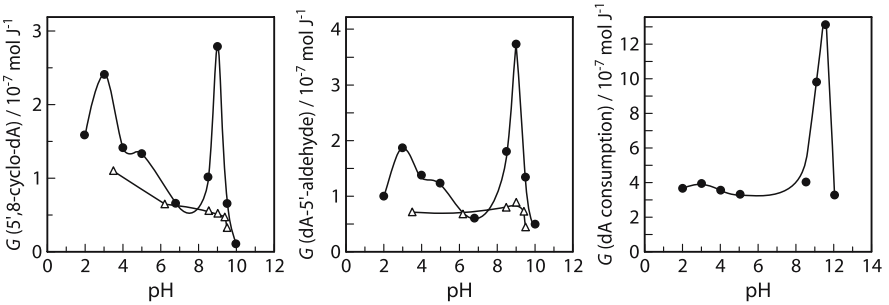


Fig. 10.1. γ-Radiolysis (*filled circles*) and electron-beam irradiation (*open triangles*) of N_2O-saturated solutions of dAdo. *G* values of the formation of the sum of the two isomers of cA (*left*), dAdo-5′-aldehyde (*middle*) and dAdo consumption (*right*) as a function of pH. Source: Wagner et al. (1999)

phosphate group (Raleigh and Fuciarelli 1985). Arguments have been put forward that this explanation is not adequate (von Sonntag 1987a). As it now turns out, the situation must indeed be much more complex. In the case of dAdo the same kind of pH dependence is observed (von Sonntag 1994). While the yield of the sum of the two AMP-derived cyclonucleotide isomers reaches a *G* value as high as 0.65×10^{-7} mol J^{-1} at its maximum (Raleigh and Fuciarelli 1985), the yield of the corresponding dAdo products is even 2.85×10^{-7} mol J^{-1} (Fig. 10.1). In addition, another product which has the *C*(5′) radical as a precursor, the dAdo-5′-aldehyde [reaction (220)], shows the same pH dependence, and their combined yields reaches a *G* value of 6.6×10^{-7} mol J^{-1} at the maximum close to pH 9.5. In fact, we deal here with a damage amplification reaction, since under the same conditions *G*(dAdo consumption) reaches a value of 13×10^{-7} mol J^{-1}, that is, twice the $^{\bullet}OH$ yield (Fig. 10.1). It is generally accepted that $^{\bullet}OH$ adds mostly to the base moiety, and thus these high values clearly rule out $^{\bullet}OH$-attack at the *C*(5′) position as the main primary process. This, however, requires that base radicals are capable to induce the formation of the *C*(5′) radicals which are the precursors of cA and 5′-CHO-dAdo. In accordance with this, the formation of cA can also be induced by $Br_2^{\bullet-}$ which is known to oxidize only the base moiety (von Sonntag 1994). Thus, besides a very small fraction of $^{\bullet}OH$ that attack the *C*(5′) position, there must be a major contribution of base radicals involved in the formation of these products.

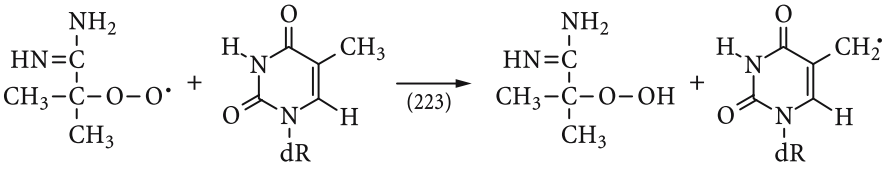
Mechanistically, this damage amplification reaction is not yet understood. Obviously, a second dAdo molecule is required for the reaction to proceed. When the termination of the radicals becomes very fast, this reaction no longer can proceed efficiently. As a consequence, the yields of 5′,8-cyclo-dAdo and 5′-CHO-dAdo drop dramatically at very high dose rate, i.e., under the conditions of pulse radiolysis (Fig. 10.1, triangles; Wagner et al. 1999). This is one of the reasons, why this reaction cannot be studied with this technique.

cG and the dGuo-5′-aldehyde are also major products in the dGuo system, but they never reach yields as high as those found with dAdo (preliminary results from the author's group), and cG is only formed in reasonable yields under

acidic conditions. At pH 3.5 where 10% of the dGuo is protonated, the cG yield is approximately halved in the presence of 5×10^{-4} mol dm^{-3} $Fe(CN)_6^{3-}$. Under such conditions, oxidation of the *C*(5′) precursor radical should be fast ($k \approx 2 \times 10^9$ dm^3 mol^{-1} s^{-1}; cf. Adams and Willson 1969), and from this observation one estimates that the intramolecular addition should be in the order of 10^6 s^{-1}. In less acidic solutions practically only the 5′-aldehyde is formed under such conditions. This is in agreement with a much lower rate of addition of α-hydroxyalkyl radicals to neutral purines (Table 10.23).

10.6 Reactions with Peroxyl Radicals

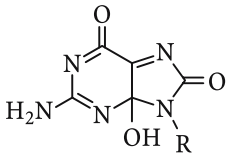
The general reactions of peroxyl radicals are discussed in Chapter 8. Here, it is recalled that peroxyl radicals are comparatively inert radicals. The reduction potential of simple alkylperoxyl radicals (~0.8 V) is considerably lower that that of Gua (1.29 V) which has the lowest reduction potential of all the nucleobases (Table 10.2). Yet, there are considerably more strongly oxidizing peroxyl radicals such as acylperoxyl radicals whose oxidation power is as high as 1.6 V, and such peroxyl radicals may stand a much better chance of reacting with the purines, notably Gua by ET. The ROO-H BDE is lower than that of typical C-H bonds, and H-abstraction reactions are therefore endothermic and thus very slow. For this reason, peroxyl radical reactions can be very selective. For the study of their reactions with substrates, it is required to reduce their bimolecular termination reactions to a minimum. This can be partly achieved by producing a very low steady-state concentration. This seems to have been realized by the slow (reaction time 25 h) thermal decomposition of 2,2′-azobis(2-methylpropinonamidine) at 40 °C (Chap. 2.4) in oxygenated solutions (Martini and Termini 1997). With Thd, these peroxyl radicals react mainly by abstracting the (most weakly bound) allylic hydrogen [reaction (223)], and in subsequent reactions the 5-methyl group is oxidized to hydroxmethyl, aldehyde and carboxylate functions.
In fact, 5-hydroxymethyluracil is a major oxidative DNA lesion, and is excreted into the urine in rather large amounts (Bianchini et al. 1996).



The same reaction has been carried out with dGuo in the context of a study of mutagenic effects of peroxyl radicals on DNA (Valentine et al. 1998). Some products have been recognized by HPLC but were not identified. Gua is not released, and there was no evidence that 8-oxo-G is among the products.

The thermolysis of dioxetanes in the presence of O_2 also yields peroxyl radicals (alkylperoxyl and acetylperoxyl), and these generate upon their reaction with dGuo mainly Z and 4-HO-8-oxo-G with only small amounts of 8-oxo-G

(Adam et al. 2001). It seems that 4-HO-8-oxo-G is not a primary product and results from an oxidation of 8-oxo-G (it is noted that the reduction potential of 8-oxo-G is only 0.74 V; Steenken et al. 2000).

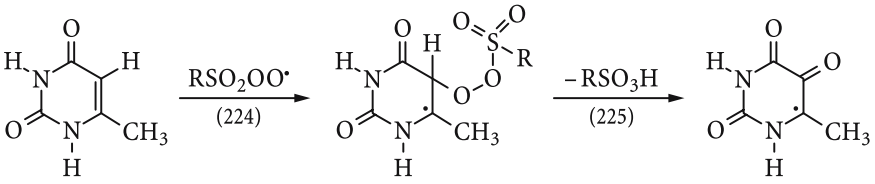


4-Hydroxy-8-oxo-guanosine (4-OH-8-oxo-G)

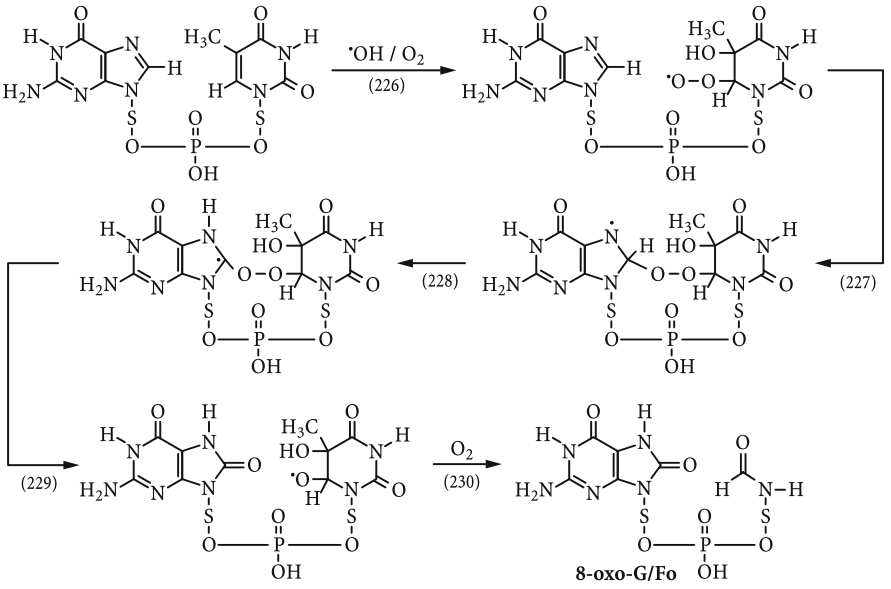
Similarly, Gua has been reported to yield 8-oxo-G albeit in only in 1% yield (relative to the peroxyl radical yield; Simandan et al. 1998). In the same study, the products from peroxyl radical reactions with other nucleobases are also reported. Ade gives rise to 8-oxo-A and 6-hydoxylaminopurine (in total 2%), while in the case of Cyt the formation of 5-hydroxy-Cyt (5%), 5,6-dihydroxyCyt (1%) and Cg (1%) are reported. Detected Thy products were the Tg (5%), $5CH_2OH$Ura (5%) and 5-hydroxy-5-methylhydantoin (1%). In this context, it is recalled that due to the slowness of the reaction of peroxyl radicals with substrates and the fast self-termination of peroxyl radicals product yields strongly depend on the rate of peroxyl radical generation. The in vivo generation of peroxyl radicals which may be much slower than typical rates of in vitro generation of peroxyl radicals could, in principle, result in higher product yields.

Mechanistically, it is difficult to see how some of these products that are well-known from $^{\bullet}$OH-induced reaction may be formed upon peroxyl-radical attack, for example, the glycols, and the question must be raised, whether these may arise from the thermal decomposition of hydroperoxides formed in preceding reactions such as reaction (223).

Alkylperoxyl radicals are not the only peroxyl radicals that react with DNA components. Sulphonylperoxyl radicals which are formed in the free-radical-induced oxidation of thiols (Chap. 7.4) also react readily with Thy by an abstraction of the allylic hydrogen (Razskazovskii and Sevilla 1996), analogous to reaction (223). With 6MeUra an interesting addition/elimination reaction has been proposed to occur [reactions (206) and (207); Razskazovskii and Sevilla 1996]. H-abstraction from the sugar moiety of nucleosides [notably *C*(1′), cf. Miaskiewicz and Osman (1994)] and in DNA from the more accessible *C*(4′)-position has also been considered.



A peroxyl-radical-induced reaction seems also to be the tandem lesion consisting of an 8-oxo-G and a formamido residue (8-oxo-G/Fo) observed upon the reaction of •OH with d(GpT) in the presence of O_2 [reactions (226)–(230); Box et al. 1993], and the same type of reactions were observed with d(GpC), d(TpG) and d(CpG) (Box et al. 1995). An ^{18}O-labeling study yielded some mechanistic information (Douki et al. 2002). The ^{18}O label is in the 8-oxo-G and to 50% also in the formamido residue. The first step is the addition of •OH to the Thy moiety with the formation of the *C*(5)- and the *C*(6)-peroxyl radicals [e.g., reaction (226)]. Upon their addition to the *C*(8)-position of the neighboring Gua moiety, an *N*-centered radical is formed [reaction (227)]. Such radicals do not react with O_2, but readily undergo 1,2-shift reactions (Chapter 7.2) [reaction (228)]. The resulting radical at *C*(8) is expected to undergo β-cleavage yielding the 8-oxoG lesion [reaction (229)]. The oxyl radical at the Thy residue will further degrade into the Fo lesion [reaction (230)]. According to this mechanism, the ^{18}O label will always be in the 8-oxo-G residue, but only in the Fo residue, when the *C*(6)-peroxyl radical is the precursor. Tandem lesions with a Z and a Fo moiety were not observed, and this may be taken as a support for the above mechanism.

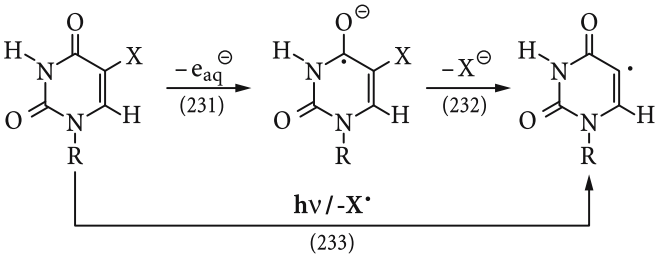


This type of damage amplification reaction is also observed in polynucleotides and in DNA (Chaps 11.2 and 12.5).

10.7 Halogenated Pyrimidines and Purines

Halogenated pyrimidines. There is a considerable interest in the reactions of 5-halourucils, notably 5BrUra. The latter can substitute Thy in DNA without

changing the viability of DNA significantly, since the van-der-Waals radii of -CH_3 and -Br are nearly identical. The incorporation of 5BrUra into DNA renders this DNA more sensitive towards ionizing and UV radiation (for reviews see Hutchinson 1973, 1985, 1987; Hutchinson and Köhnlein 1980; von Sonntag 1987a). The main reason for this sensitizing effect is thought to be due to the formation of the reactive uracil-5-yl radical formed under both conditions [reactions (231)-(233); for spin-trapping of this radical see Hedrick et al. 1982].



Upon photolysis, there are two reactive species formed side by side, the vinylic uracilyl radical and Br•. The latter has oxidizing properties (E^7 = 1.66 V; Wardman 1989), and in DNA it can oxidize neighboring G and A moieties (Wojcik et al. 2003). The quantum yield of photodecomposition of 5BrUra is only 1.8×10^{-3}, but increases dramatically upon deprotonation (Campbell et al. 1974). In the presence of MeOH as an H-donor, it also increases substantially above 2 mol/l MeOH. It has been speculated that above this concentration cage reactions come into play. Reported rate constants for the reaction of Br• with MeOH are 5×10^4 dm^3 mol^{-1} s^{-1} in water and 1×10^6 dm^3 mol^{-1} s^{-1} in acetonitrile (Neta et al. 1988).

The haloracil radical anion eliminates the halide ion [reaction (232); for a quantum mechanical calculation, albeit not taking solvation into account see Sommerfeld 2001]. In the case of 5IUra•⁻ and 5BrUra•⁻, this reaction is very rapid and occurs on the nanosecond time scale (Table 10.25), and protonation cannot intercept these species at pH 3 (Bhatia and Schuler 1973; Wagner et al. 1974). At 77 K, however, 5BrUra•⁻ is sufficiently long-lived to be observed by EPR (Riederer et al. 1978), and only at 155 K it decays according to reaction (232) (Sevilla et al. 1974). The elimination of Cl^- from 5ClUra•⁻ is already sufficiently slow for other reactions, e.g., a protonation reaction, to be able to compete (Wagner and Schulte-Frohlinde 1975; Burr et al. 1976). With 5FUra•⁻ the rate of F^--elimination is now so slow that even at the low dose rates of γ-radiolysis F^--elimination remains incomplete (Bansal et al. 1972). Details are as yet not known, but it is conceivable that 5FUra•⁻ is protonated at carbon (see above), and the subsequent combination reactions of the ensuing radicals no longer release F^-.

5BrUra•⁻ is not only formed in the reaction of e_{aq}^- with 5BrUra, but other reducing radicals produce this intermediate as well (Zimbrick et al. 1969; Bansal et al. 1972; Görner 1993). Possibly, more important for the sensitizing effect in DNA is the fact that the radical anions of Thy and Ade can also transfer an electron to 5BrUra (Nese et al. 1992). In model systems this is no longer possible with Gua (for a study of the ET in 5BrUra-substituted oligonucleotides see Fuciarelli et al.

Table 10.25. Rate constants of halide elimination from the radical anions of 5-halouracils

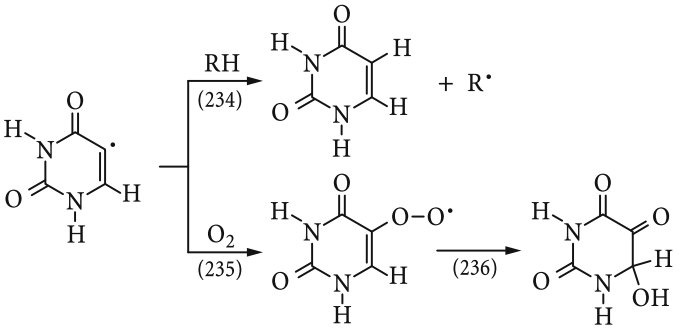
5-Halouracil	Rate constant/s^{-1}	Reference
5IUra	4×10^8	Rivera and Schuler (1983)
5BrUra	1×10^8	Rivera and Schuler (1983)
5ClUra	1.4×10^5 9×10^4	Rivera and Schuler (1983) Wagner and Schulte-Frohlinde (1975)
5FUra	Slow	Bansal et al. (1972)

Table 10.26. Compilation of rate constants of ET from electron adducts and their protonated forms of nucleobases and nucleosides. (Nese et al. 1992)

Substrate	Reaction	k/$dm^3\ mol^{-1}\ s^{-1}$	Reference
Thy	$Thy^{\bullet -}$ + 5BrUra	1.1×10^9	Adams and Willson (1972)
	$Thy^{\bullet -}$ + Cyt	1.7×10^9	Nese et al. (1992)
	$Thy^{\bullet -}$ + Guo	5.0×10^8	Nese et al. (1992)
		7.0×10^7	Nese et al. (1992)
Thd	$ThdH^{\bullet}$ + 5BrUra	2.3×10^7	Nese et al. (1992)
	$Thd^{\bullet -}$ + 5BrUra	7.2×10^8	Nese et al. (1992)
Ado	$AdoH^{\bullet}$ + 5BrUra	3.5×10^8	Adams and Willson (1972)
		2.0×10^8	Nese et al. (1992)
Cyt	$CytH^{\bullet}$ + 5rBrUra	$< 5 \times 10^7$	Adams and Willson (1972)
	$CytH^{\bullet}$ + Thy	2.0×10^7	Nese et al. (1992)
		2.0×10^6	Nese et al. (1992)
Guo	$GuoH^{\bullet}$ + 5BrUra	No transfer	Nese et al. (1992)

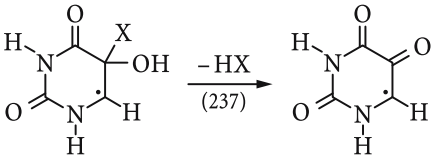
1994). It is discussed above that the nucleobase radical anions can be protonated at a heteroatom and/or carbon. Only the heteroatom-protonated species retains reducing properties, and thus the rate of protonation at carbon determines whether or not an ET to 5BrUra is observed under the given condition. Protonation at carbon is especially fast in the case of Guo, and for this reason an ET to 5BrUra was not observed (Nese et al. 1992). A compilation of the rate constants for such ET reactions is found in Table 10.26. As can be seen from this table, the radical anions transfer an electron to 5BrUra at practically diffusion-controlled rates, while the heteroatom-protonated species react two orders of magnitude more slowly.

The Ura-5-yl radical is a vinyl-type radical. It readily adds to, e.g., its parent, 5BrUra ($k = 2.7 \times 10^8$ dm^3 mol^{-1} s^{-1}; Rivera and Schuler 1983), but vinyl radicals are also good H-abstractors [cf. reaction (234)] and typically react with, e.g., 2-PrOH in the rage of 2×10^5 to 2.4×10^7 dm^3 mol^{-1} s^{-1}, depending on the substituent (Mertens 1994; Mertens and von Sonntag 1994a).



Their corresponding peroxyl radicals have the unusual property of absorbing strongly in the visible (Chap. 8.2). The Ura-5-peroxyl radical formed in reaction (235) also absorbs in the visible ($\lambda_{max} = 570$ nm), and it has been shown by pulse radiolyis that it decays rapidly (6×10^4 s^{-1}), apparently unimoleculary (Mertens and von Sonntag 1994a). The resulting product is isodialuric acid [Gilbert and Schulte-Frohlinde 1970; reaction (236)], and it had already postulated by these authors that a unimolecular process might be involved in the formation of this product.

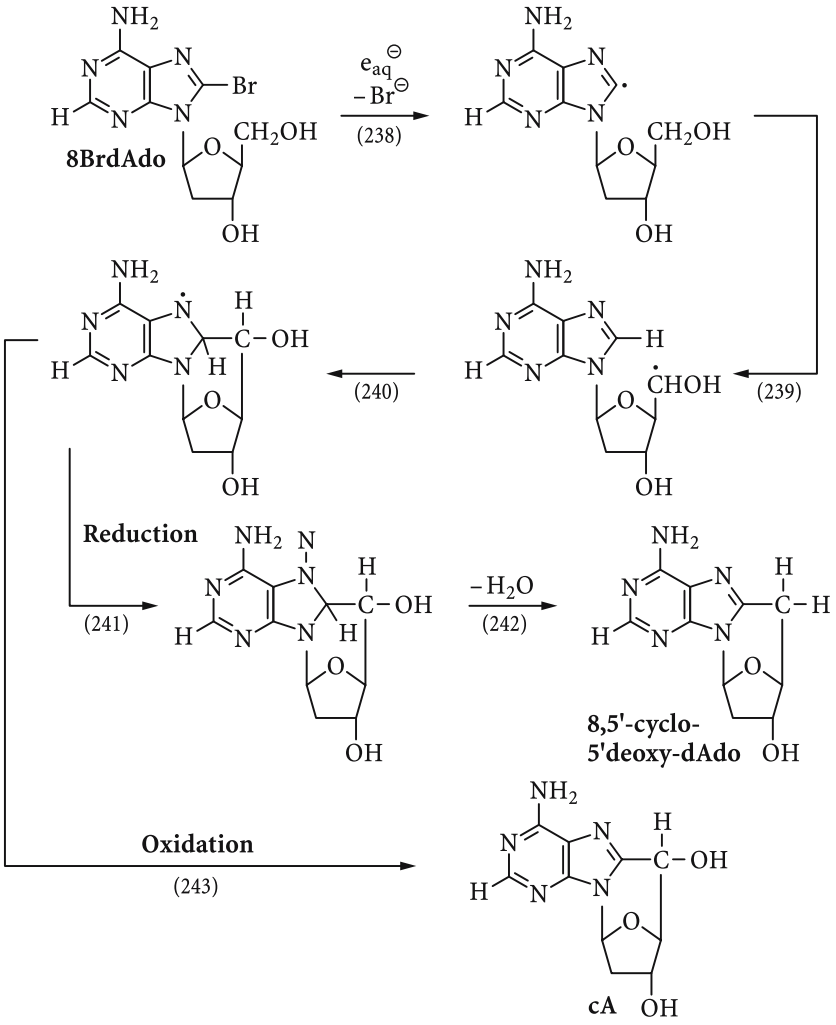
Addition of •OH to C(5) in 5-haloruacils leads to a rapid release of HX [reaction (237); $k \geq 10^6$ s^{-1}; Bansal et al. 1972; Neta 1972; Patterson and Bansal 1972; Mori et al. 2001] as is typical for geminal halohydrines (Köster and Asmus 1971; Mertens and von Sonntag 1994b; Dowideit et al. 1996).



The resulting radicals have only weakly oxidizing properties and react with TMPD with a rate constant of 2×10^8 dm^3 mol^{-1} s^{-1} (Mori et al. 2001).

Halogenated purines. 8BrdAdo has been used to study some of the steps in the cyclization reaction that yields the purine cyclonucleosides/tides (Flyunt et al. 2000; Chatgilialoglu et al. 2003). Upon the reaction of e_{aq}^-, the very reactive vinylic radical at *C*(8) is formed [reaction (238); $k = 1.6 \times 10^{10}$ dm^3 mol^{-1} s^{-1}] which undergoes rapid H-abstraction from the hydroxymethyl group at *C*(5′) [reaction (239)]. In neither of these reactions are radicals formed which strongly absorb in the accessible UV/Vis range. Upon addition of the *C*(5′) radical to *C*(8) [reaction (239); $k = 1.6 \times 10^5$ s^{-1}], an aminyl radical is formed whose UV/Vis spectrum is

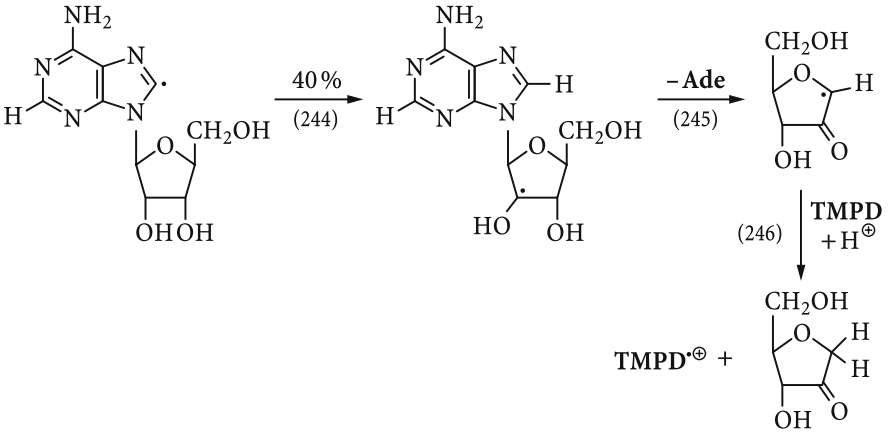
similar to that of the *C*-protonated electron adduct of dAdo. Subsequent redox reactions with $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ gives rise to the observed products, cA and 8,5′-cyclo-5′-deoxy-dAdo [reactions (241)-(243)].



The rate constants for the *C*(5′) radical with O_2 ($k = 1.8 \times 10^9$ dm^3 mol^{-1} s^{-1}) and $Fe(CN)_6^{3-}$ ($k = 4.2 \times 10^9$ dm^3 mol^{-1} s^{-1}) are close to diffusion controlled. In contrast, the aminyl radical reacts with O_2 reversibly and is oxidized by $Fe(CN)_6^{3-}$ markedly slower ($k = 8.3 \times 10^8$ dm^3 mol^{-1} s^{-1}). With $Fe(CN)_6^{3-}$ as the oxidant, the (5′*R*):(5′*S*) diastereoisomeric ratio of cA is 6.

In the reactions of e_{aq}^- with 8BrAdo are markedly different (Chatgilialoglu et al. 2004). The first step is the same and equally fast, but the *C*(8) radical now not only abstracts the *C*(5′)-H (~60%) but also the *C*(2′)-H (~40%). The *C*(5′) radical adds considerably more slowly to the *C*(8)-position as compared to the

8BrdAdo system ($k \approx 2.3 \times 10^4$, von Sonntag 1987b; $k \approx 1 \times 10^4\ s^{-1}$, Chatgilialoglu et al. 2004), possibly due to conformational differences. Thus, the *C*(5′) radical has a much longer lifetime, and as a consequence high yields of the 5′-aldehyde are formed in the presence of an oxidant such as TNM or O_2 (von Sonntag 1987b). H-abstraction of the *C*(2′)-H leads to the release of Ade [reactions (244) and (245); Chatgilialoglu et al. 2004]. The oxidizing radical formed upon Ade release is monitored by TMPD [reaction (246)], and from a computer analysis of the data a rate constant of $k_{245} = 1.1 \times 10^5\ s^{-1}$ and $k_{246} = 4.6 \times 10^9\ dm^3\ mol^{-1}\ s^{-1}$ has been arrived at (in the original paper, it has been suggested that TMPD is oxidized by the precursor radical cation, but this will deprotonate too rapidly to undergo this reaction).



The Ado *C*(2′) radical has been generated separately in a photolytic experiment using an adequately substituted Ado derivative, and from a competition of Ade release and reduction by GSH $k_{GSH}/k_{245} = 4.3\ dm^3\ mol^{-1}$ has been arrived at. Based on the assumption that k_{GSH} should be around $10^6\ dm^3\ mol^{-1}\ s^{-1}$ a reasonable (within a factor of 2) agreement with the above k_{245} has been obtained. Considering, however, that α-hydroxyalkyl radicals react in aqueous solution at least 50 times faster with thiols (Chap. 7.4), the rate of Ade release may even be much faster then estimated from the above-mentioned computer analysis of the data.

A detailed study on the reaction of 8BrGuo with reducing radicals, notably e_{aq}^- but also with other reducing radicals, has shown that in these reactions it behaves differently from 5BrUra and 8BrdAdo (Ioele et al. 2000). In this case, a comparatively long-lived ($k = 5 \times 10^4\ s^{-1}$) intermediate absorbing at around 600 nm is formed, and in H_2O vs D_2O, there is a strong kinetic isotope effect of $k_H/k_D = 8.0$ in its decay. In the presence of *t*BuOH, equal amounts of Br^- and Guo are formed, and when the reaction is carried out in D_2O, deuteration occurs at *C*(8). Based on spectral similarities, it is suggested that on the way to these products the $G^{\bullet+}$ or $G^{\bullet}$ are intermediates, but it is difficult to see wherefrom the reduction equivalent should come. It is not expected that these intermediates are capable of abstracting an H atom from *t*BuOH with a rate constant of about $10^4\ dm^3\ mol^{-1}\ s^{-1}$ as would be required for a complete reduction of the inter-

mediate by *t*BuOH under the given conditions. Thus, the suggested mechanism must remain tentative. A way out of this dilemma would be if the radical anion first protonated at a heteroatom and subsequently at *C*(8) (these are typical purine reactions, see above, and the latter step would be connected with such a large KIE, see above). The resulting *N*(9) radical would have to undergo $Br^{\bullet}$ loss yielding the observed product Guo and the right isotope labeling. Elimination of $Br^{\bullet}$ in β-fragmentation reactions is common.

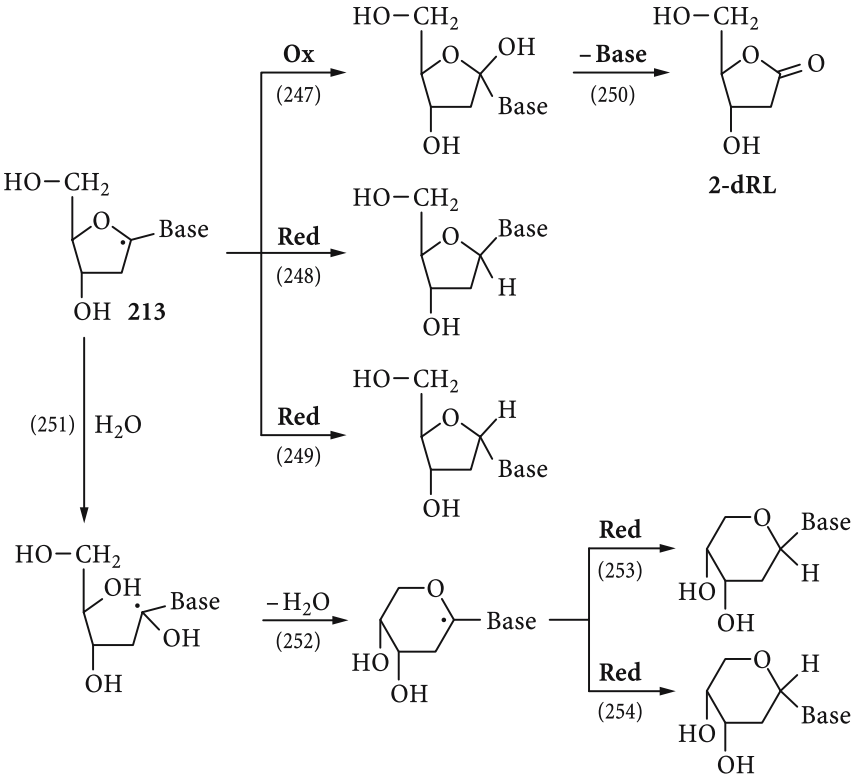
10.8 Reactions of Sugar-Derived Radicals in Nucleosides and Nucleotides

The bond dissociation energies of the various C–H bonds in the 2-deoxyribose moiety have been calculated on the basis of *N*(1)–H BDE in dGuo (380 kJ mol^{-1}; Steenken et al. 2001). They are compiled in Chap. 4, Table 4.6.

The *C*(1′)-H is the most loosely bound hydrogen, followed by *C*(4′)-H. In H-abstraction reactions, these positions are the most likely ones to be attacked be reactive free radicals, while those bound to *C*(2′) are the most unlikely ones. This argument only holds when other parameters, e.g. steric factors and acessibility as in DNA (Chap. 12.2), can be neglected. In the *ribo*-series, the *C*(2′)–H is, of course, again only loosely bound, and many striking differences between the free-radical chemistry of 2′-deoxynuleosides and nucleosides and more noticeably in the corresponding homopolymers may be due to this difference (Chap. 11.2).

10.8.1 Isomerization at *C*(1′) and *C*(4′)

The *C*(1′) radical assumes a close to planar conformation. Upon subsequent reduction the α- and β-isomers are likely to be formed in approximately equal yields [reactions (248) and (249)].



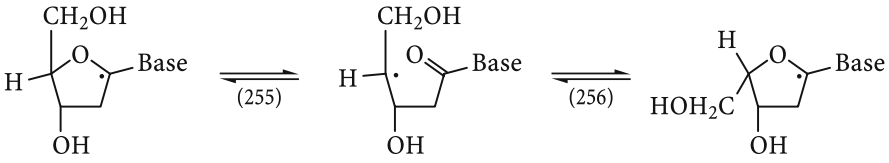
When the starting material is the nucleoside, the reformation of the β-form cannot be monitored, but in the case of dAdo (Mariaggi et al. 1979) and dGuo (Berger and Cadet 1985) the α-form as well as the pyranose forms have been detected. To account for the latter, one has to assume that during the radical lifetime hydrolysis of the *O*-glycosidic linkage occurs [reaction (251)] and reclosure to the pyranose forms takes place [reaction (252)] prior to a subsequent reduction [reactions (253) and (254)]. A rapid hydrolysis of the *O*-glycosidic linkage is a well-documented process in the free-radical chemistry of disaccharides (Kochetkov et al. 1965, 1968; Dizdaroglu and von Sonntag 1973; von Sonntag et al. 1976; Adam 1977; Zegota and von Sonntag 1977), but that the reverse reaction must also be fast has no reported analogy in these systems.

The *C*(1′) radical can also be produced upon UV-photolysis of the corresponding *tert*-butylketone (see below; Goodman and Greenberg 1996; Chatgilialoglu et al. 2000). In the presence of H-donors such as thiols or 1,4-cyclohexadiene, the dUrd-1′-yl radical is reduced into a 3:2 mixture of the β- and α-2′-deoxynucleosides [cf. reactions (248) and (249)]. The rate constant of H-donation by thiols varies only little (mercaptoethanol: 2.3×10^6; cystein: 2.9×10^6; glutathione: 4.4×10^6 $dm^3\ mol^{-1}\ s^{-1}$) and so does the anomeric ratio of the product, dUrd (Chatgilialoglu et al. 2000).

In the case of dGuo an isomerization at *C*(4′) has also been observed. In a preliminary communication this product had been assigned to the β-anomer

Table 10.27. γ-Radiolysis of Thd in N_2O- and N_2O/O_2-saturated solutions. *G* values (unit: 10^{-7} mol J^{-1}) of altered sugars and released Thy. (Dizdaroglu et al. 1976)

Product	N_2O	N_2O/O_2
2,5-Dideoxy-pentos-4-ulose	0.01	Absent
2,4-Dideoxy-pentadialdose	0.02	Absent
2,4-Dideoxy-pentos-3-ulose	0.03	Absent
2,3-Dideoxypentos-4-ulose	0.1	Absent
2-Deoxy-pentos-4-ulose	0.1	0.2
2-Deoxyribonolactone, 2-dRL	0.02	0.07
2-Deoxy-tetrodialdose	Absent	0.03
Thy	≤0.2	≤0.4



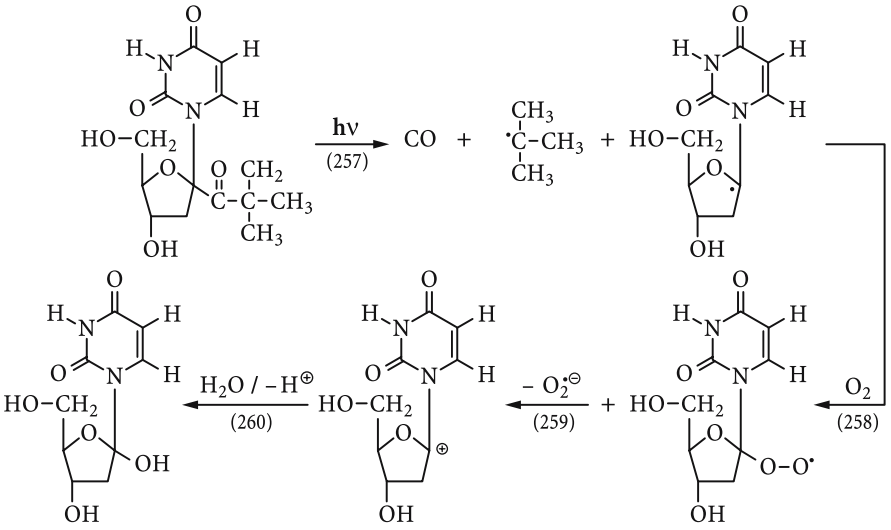
(Berger and Cadet 1983a), but later (Berger and Cadet 1985) to the α-anomer (Table 10.13). The formation of the α-anomer poses the question, how two centers can be isomerized in subsequent reactions. It is well established that in carbohydrates the *C*(1′)-type radical can undergo β-scission (for a review see von Sonntag 1980). If this reaction is reversible the isomerization of two centers is understood.

10.8.2 Oxidation at *C*(1′)

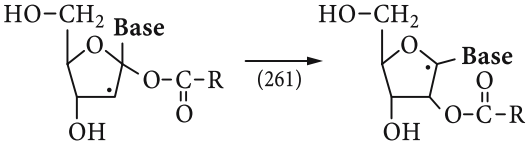
The •OH-induced oxidation of Thd at *C*(1′) leads to the formation of 2-dRL and the concomitant release of Thy [reactions (247) and (250); Dizdaroglu et al. 1976]. In the absence of an oxidant, 2-dRL is formed only in low yields (Table 10.27), and disproportionation reactions with other radicals present have to account for its formation. In the presence of O_2, its yield is considerably increased. This raises the question as to how these reactions may proceed.

This requires a specific generation of the *C*(1′) radical. To this end, the corresponding *tert*-butylketone derivative has been synthesized (Goodman and Greenberg 1996; Chatgilialoglu et al. 1998; Chatgilialoglu and Gimisis 1998). Upon UV photolysis, it looses CO and a *tert*-butyl radical [reaction (257); for

their EPR spectra see Chatgilialoglu et al. 1998]. In the absence of an oxidant, the radicals disproportionate and dimerize. Disproportionation yields α-/β-dUrd and Ura/2-dRL (via the hydrolysis of 1′,2′-dehydro-dUrd) in ~15% yields each pair (Goodman and Greenberg 1996). In a laser flash photolysis experiment, the reaction of the *C*(1′) radical with O_2 [reaction (258)] was determined at $k = 1 \times 10^9$ dm^3 mol^{-1} s^{-1} (Chatgilialoglu et al. 1998). In the presence of TNM, the nitroform anion is formed with a rate constant of 1.5×10^4 s^{-1} (Emanuel et al. 1999). This has been taken as evidence for the rapid release of $O_2^{\bullet -}$ from the *C*(1′)-peroxyl radical [reaction (259)]. In contrast, based on thiol scavenging experiments, it has been calculated that the rate of $O_2^{\bullet -}$ elimination can only be in the order of 1 s^{-1} (Tallman et al. 1998).



In this context, it is worth mentioning that the *C*(1′)-substituted *C*(2′) radical undergoes rapid β-(acyloxy)alkyl rearrangement [reaction (261)], whereby a *C*(1′)-type radical is also formed (Gimisis et al. 1995, 1998).

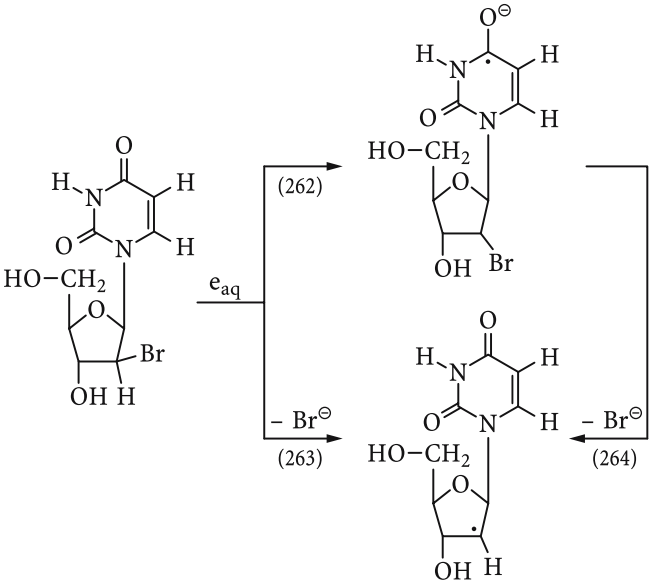


Being a strongly reducing radical, the *C*(1′) radical is also readily oxidized by Fe^{3+} and Cu^{2+}. Rate constants for these reactions have been determined at ~1×10^8 dm^3 mol^{-1} s^{-1} and 7.9×10^7 dm^3 mol^{-1} s^{-1}, respectively (Chatgilialoglu et al. 2000).

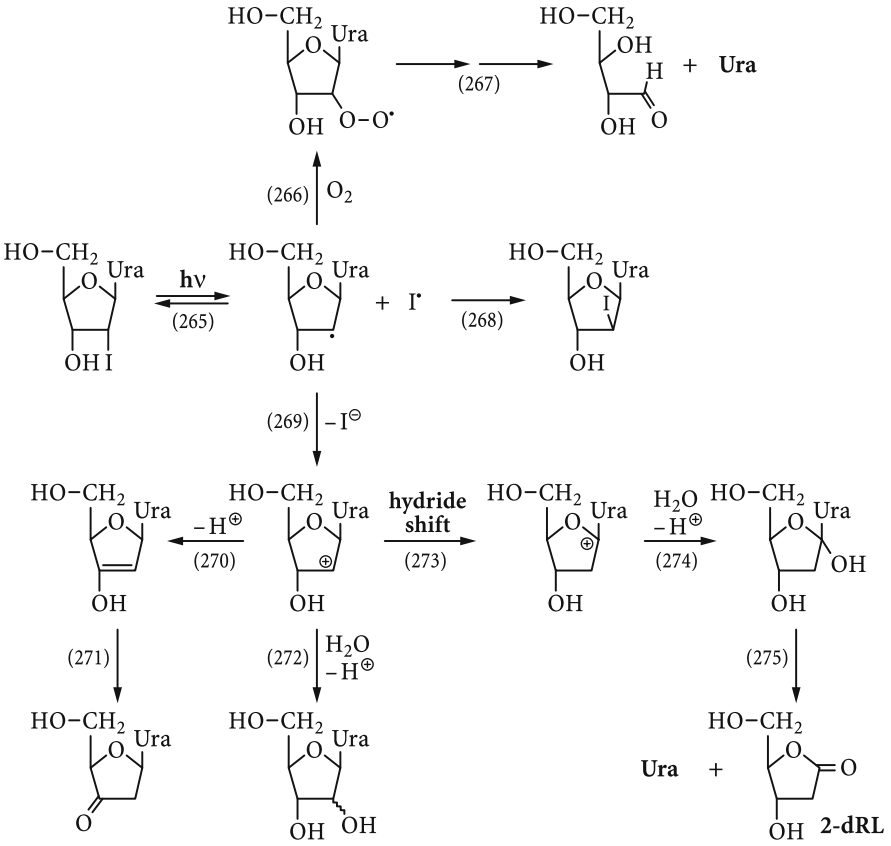
The *C*(1′)-radical is also an intermediate in the free-radical-induced cylisation of 6-(2,2-dibromovinyl)uridine (Gimisis and Chatgilialoglu 1996).

10.8.3
Oxidation at *C*(2′)

The *C*(2′) radical has been generated with some selectivity by reacting 2′BrdUrd with e_{aq}^- [reaction (263)-(264); Hissung et al. 1981b].



In the presence of O_2, it is converted into the corresponding peroxyl radical [reaction (266)]. The bimolecular decay of this peroxyl radical with the other peroxyl radicals present is this system leads to erythrose [reaction (267)] in 15% yield, i.e., the β-fragmentation reaction of a short-lived oxyl radical intermediate is of minor importance.

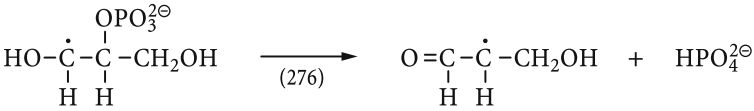


If the peroxyl radical chemistry of other alkyl radicals is any guide formation of hydroxyl and carbonyl functions at the *C*(2′) position are much more likely products (Chap. 8.8). This view is supported by the observation that in Z-form DNA intrastrand H-abstraction of the 2′β-hydrogen by the dUrd-5-yl radical results in 2′-hydroxylation in the presence of O_2 (Kawai and Saito 1999), but the above-mentioned erythrose lesion is also observed in DNA (Dizdaroglu et al. 1977), in dsODNs (Sugiyama et al. 1997) and may even become the major *C*(2′)-derived lesion (Sugiyama et al. 1993). Important information as to the fate of the *C*(2′) radical has been obtained from a study on the photolyis of 2′IdUrd (Sugiyama et al. 1995). The major products (for some additional products, see References) and the suggested pathways for their formation are shown in reactions (265)–(275).

The primary photolytic step is the homolytic scission of the C-I bond [reaction (265)]. The resulting radicals may recombine [reactions (–265) and (268)]. An ET within the solvent cage has also been suggested [reaction (269)]. Alternatively, one may consider the direct formation of the carbocation and iodide ion as a second photolytic pathway. Interestingly, there seems to be a rapid hydride shift [reaction (273)]. Deprotonation at *C*(1′), analogous to reaction (270), and

rapid hydrolysis of the ensuing enol ether [cf. Janik et al. (2000)] would be an alternative pathway. The observed pentose-nucleosides that are suggested to be formed in reaction (245) may also have peroxyl radicals as precursors (see the above discussion).

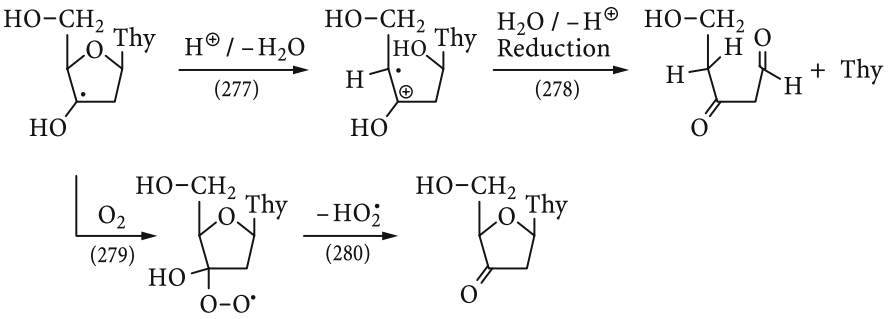
Attack at *C*(2′) in 3′-*ribo*-nucleotides is followed by a rapid release of phosphate as can be deduced from corresponding model systems such as the glycerophosphates (Samuni and Neta 1973; Steenken et al. 1974; Schuchmann et al. 1995) [reaction (276); $k > 10^6\ s^{-1}$; Schuchmann et al. 1995; see also Chap. 6.9].



In *ribo*-nucleosides, the *C*(2′) radical releases very rapidly the base (Chatgilialoglu et al. 2004). Details have been discussed in the paragraph on halogenated bases.

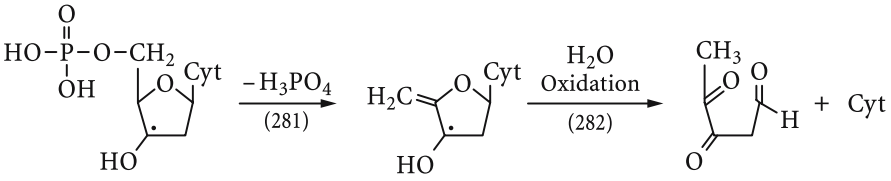
10.8.4 Oxidation at *C*(3′)

The Thd *C*(3′) radical is an α-hydroxyalkyl-β-alkoxyl radical that can rearrange and yields after reduction 2,4-dideoxy-pentos-3-ulose, whereby Thy is released (Dizdaroglu et al. 1976) [reactions (277) and (278); for the yield, see Table 10.27].

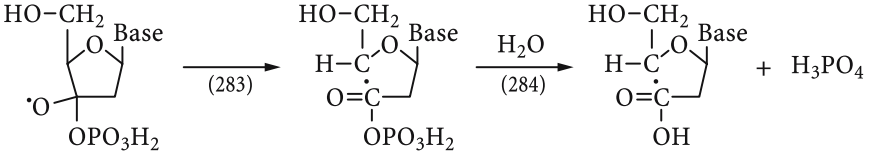


In the presence of O_2, formation of 3-keto-dThd is the most likely process [reactions (279) and (280)]. Since the peroxyl radical formed in reaction (279) is closely related to that derived from 2-PrOH, the rate of $HO_2^{\bullet}$-elimination is expected to be close to $650\ s^{-1}$ (cf. Bothe et al. 1977).

An interesting route has been observed with dCMP (Schuchmann et al. 1983). The formation of the allylic radical upon elimination of phosphoric acid seems to be the driving force for reaction (281). Cyt is released in the subseqent reactions (282). The suggested mechanism has been supported by experiments in D_2O.



H-abstraction at *C*(3′) in 3′-nucleotides gives rise to an α-phosphatoalkyl radical (the phosphatoalkyl group in α-position favors the rate of H-abstraction less than an OH group; Schuchmann et al. 1995). From a study on the •OH-induced reactions of trimethylphosphate, it has been concluded that a hydrolysis of the $^{\bullet}CH_2OP(O)(OCH_3)_2$ radical must be very slow at pH 7 and is just noticeable at high pH (von Sonntag et al. 1972). In the presence of O_2, the alkyl group is fully degraded (formation of dimethylphosphate in full yield; Schuchmann and von Sonntag 1984). The rapid formation of acids ($k = 0.3 + 2.3 \times 10^4$ [OH^-] s^{-1}) has been attributed to the hydrolysis of the anhydride of formic acid and dimethylphosphoric acid (for the hydrolysis of other anhydrides of formic acid see Leitzke et al. 2003). Much closer to the question of the potential reactions of the *C*(3′) radical is a study on triisopropyl phosphate (Schuchmann et al. 1984c). In the presence of O_2, ~50% of •OH gives rise to the formation of diisopropylphosphate, and acetic acid is an important product (Schuchmann et al. 1995). This points to the formation of the anhydride of acetic acid and diisopropylphosphoric acid as an intermediate whose precursor must be an oxyl radical. Translated to the *C*(3′)-O• formed upon the bimolecular decay of the *C*(3′)-OO• radicals of nucleotides, fragmentation of the *C*(3′)-*C*(4′) bond will occur [reaction (283)], and the anhydride function in will subsequently hydrolyze [reaction (284)]. Of course, there are further decay routes, but the reaction sequence shown here must certainly be of a major importance.



Oxidation of α-phosphatoalkyl radicals by TNM leads first to an adduct which subsequently decays thereby releasing NF^- (Schuchmann et al. 1995). Nitroaromatic sensistizers form also adducts albeit more slowly than TNM. Oxidation of α-phosphatoalkyl radicals by $Fe(CN)_6^{3-}$ is only moderately fast.

Phosphate release yields from some 3′- and 5′-mononucleotides are compiled in Table 10.28. The data in N_2O-saturated solutions indicate that phosphate release from the 3′-position is about twice as efficient than that from the 5′-position. Correcting for the fact that under O_2 the •OH yield is about halved, there is a protecting effect for 3′-AMP 3′-dAMP and 3′-GMP but an enhancement in the case of 3′-CMP and 3′-UMP. For the 5′-nucleotides, there is always an increase

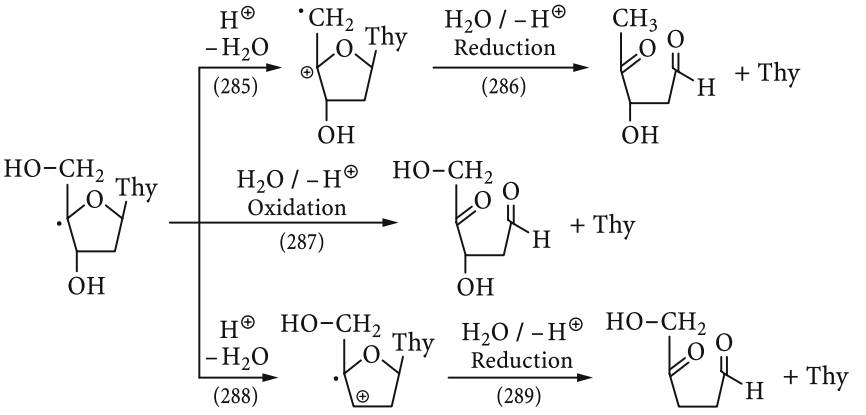
Table 10.28. *G* values (unit: 10^{-7} mol J^{-1}) of inorganic phosphate release in the γ-radiolysis of aqueous solutions of some 3′- and 5′-monoucleotides (Raleigh et al. 1974). Data from Greenstock and Shierman (1975) are given in brackets

Nucleotide	N_2O		O_2	
	3′	5′	3′	5′
AMP	1.02 (1.0)	0.36 (0.29)	0.22	0.29
GMP	0.45	0.24 (0.22)	0.14	0.22
CMP	0.70 (0.67)	0.37 (0.38)	0.72	0.43
UMP	0.75 (0.76)	0.44 (0.45)	0.80	0.48
TMP	0.86	0.37 (0.35)	–	–
dAMP	0.81	0.34 (0.34)	0.13	0.31
dGMP	(0.42)	(0.23)		
dUMP		(0.37)		
dCMP		(0.37)		

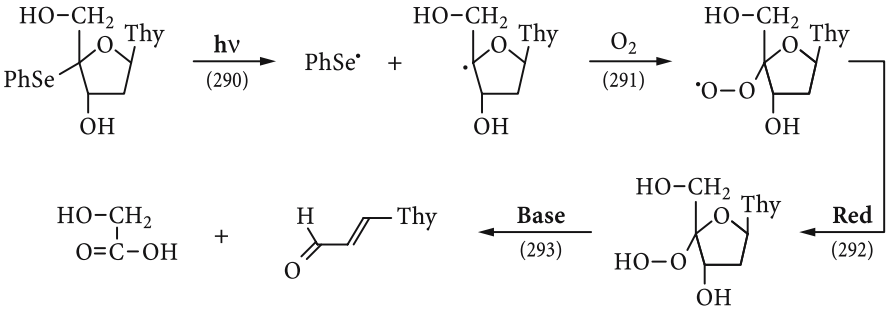
(per •OH) when O_2 is present. Lacking an obvious trend, it is premature to come up with a convincing mechanistic proposal. In the *ribo*-series, the *C*(2′) radicals certainly contribute to phosphate release as discussed above.

10.8.5 Oxidation at *C*(4′)

Some of the sugars that are formed in the radiolysis of Thd also have the *C*(4′) radical as precursor (Dizdaroglu et al. 1976) (for a quantum-chemical study of conformation of the *C*(4′) radical and hyperfine coupling constents see Parr and Wetmore 2004). The water elimination reactions as depicted in reactions (285) and (288) are generally proton-catalyzed. Yet, the ensuing products are also observed in neutral solution. Their yields are given in Table 10.27.

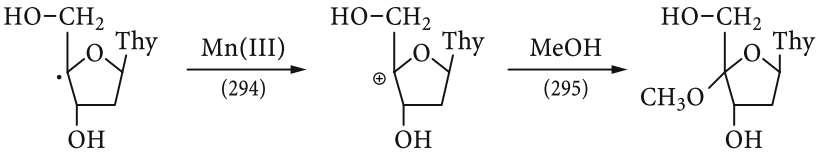


The *C*(4′) radical has been generated specifically photolytically in the presence of O_2 [reactions (290)–(293); Giese et al. 1995].



The hydroperoxide (two isomers) is formed in high yield under these conditions (the nature of the reduction equivalent that is required for this process is as yet not known), and subsequent treatment with a base gives rise to glycolic acid and the base propenal [Grob fragmentation; reaction (292); yields near 90%].

In the absence of O_2 and in the presence of Mn(III) acetate, the *C*(4′) radical is oxidized to the corresponding radical cation which in MeOH gives rise to the acetal [reactions (294) and (295); Beyrich-Graf et al. 1998].



To study the effect of a phosphate group at *C*(5′), D-ribose-5-phosphate has been investigated in some detail as a model system (Stelter et al. 1974, 1975a,b, 1976). The phosphate group is a much better leaving group than the OH group, and its elimination does not require proton catalysis. The data compiled in Table 10.29

Table 10.29. Products and their *G* values (unit: 10^{-7} mol J^{-1}) from γ-irradiated solutions of D-ribose-5-phosphate. (Stelter et al. 1976)

Product	N_2O	N_2O/Fe(II)	N_2O/Fe(III)	N_2O/H_2O_2	N_2O/O_2
Inorganic phosphate	1.35	n.d.	n.d.	1.45	0.62
Pentodialdose	0.23	0.47	0.65	0.38	0.10
2-Hydroxy-4-oxoglutaraldehyde	0.07	Absent	Absent	0.07	Absent
5-Deoxypentos-4-ulose	0.10	0.83	0.44	0.1	Absent
3-Oxoglutaraldehyde	0.06	Absent	Absent	0.06	Absent
Tetrodialdose	Absent	Absent	Absent	Absent	0.42
Formic acid	Absent	Absent	Absent	Absent	0.43
Minor phosphate-free products	0.1	Absent	0.12	0.06	0.09

n.d., Not determined

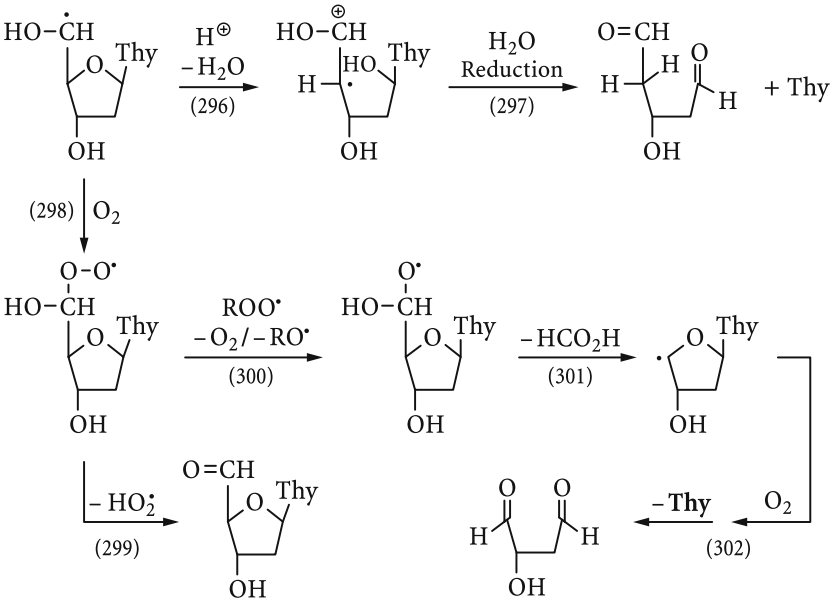
substantiate the view expressed above. They will not be discussed in more detail.

In DNA, H-abstraction at *C*(4′) leads to strand breakage and the same products as reported here are formed in the course of this process (Chap. 12.4). In this reaction, an alkene radical cation/phosphate anion pair is formed. The dynamics of this reaction has been studied in some detail with the help of adequately substituted model systems (Crich and Huang 2001).

10.8.6 Oxidation at *C*(5′)

The formation of purine 5′,8-cyclonucleosides and -cyclonucleotides and pyrimidine 5′,6-cyclco-nucleosides are a typical product of an oxidation at *C*(5′). Because it involves an addition of an alkyl radical to the base moiety, this reaction has already been discussed in Section 5.3.1. The dAdo *C*(5′) radical was generated specifically by reacting 8BrdAdo with e_{aq}^-. The ensuing reactions are discussed in Section 7.2.

According to the study on Thd already mentioned above (Dizdaroglu et al. 1976), the release of Thy is connected with the formation of 2,4-dideoxypentodialdose [reactions (262) and (263)].



In the presence of O_2, the *C*(5′) radical is converted into the corresponding peroxyl radical [reaction (298)]. Upon its bimolecular decay with other peroxyl radicals present, the oxyl radical is formed [reaction (296)]. An analogous oxyl radical has been produced by thermally decomposing the *tert*-butyl ester of thymidine-5′-carboxylic acid (Montevecchi et al. 2004). These oxyl radicals undergo ready β-fragmentation [reaction (301)], and the most pronounced products of this reaction are 2-hydroxytetrodialdose and Thy [reaction (302); Dizdaroglu et al. 1976]. Interestingly, the $HO_2^{\bullet}$-elimination [reaction (299)] is not sufficiently fast to compete effectively at pH 7. For yields, see Table 10.27.

10.8.7 Base Release

The release of unaltered bases is a general phenomenon of the free-radical-induced reactions of DNA and its constituents. The process is multi-phasic. Typically, a fraction is set free on the time-scale of the free-radical reactions or very shortly afterwards (Table 10.30). Mechanistically, this could mean that a base can already be eliminated at the free-radical stage (for an example see above) or that a resulting non-radical product is very unstable. The determination of the released bases (e.g., by HPLC) requires a couple of minutes. Thus, a fast hydrolyzing intermediate cannot be distinguished experimentally from processes occurring at the free-radical stage. However, the slow component is certainly due to unstable non-radical products. In the course of base release, the altered sugars that gave rise to this instability of the *N*-glycosidic linkage are set free. Their determination yields valuable information as to the structures of these labile products and may even provide a clue as to the mechanism of their formation.

Table 10.30. γ-Radiolysis of N_2O/O_2-saturated aqueous solutions of 2′-deoxynucleosides (2×10^{-3} mol dm^{-3}). *G*(base release) (unit: 10^{-7} mol J^{-1}) immediately after irradiation and after heating for 3h at 60 °C. (Wagner and von Sonntag, unpubl. results)

2′-Deoxynucleoside	Immediately	After 3 h at 60 °C
dAdo	0.37	0.57
dGuo	0.16	0.27
dCyd	0.20	0.51
Thd	0.14	0.45

Table 10.31. *G* (base release) from nucleosides and nucleotides γ-irradiated in aqueous solution

Substrate	Saturating gas	*G* value	Substrate	Saturating gas	*G* value
Thd	N_2O	0.2[a]	Urd	N_2O	0.66[b]
	N_2O	0.14[b]	UMP	Air	0.28[d]
	N_2O/O_2	0.4[a]	Ado	N_2O	1.2[b]
	N_2O/O_2	0.14[b]	ADP	Air	0.35[e]
dCyd	N_2O	0.79[b]	Ado-3′,5′-P	N_2O	0.2[b]
dAdo	N_2O	0.43[b]		N_2O/O_2	0.39[b]
	N_2O/O_2	0.54[b]	Inosine	N_2O	0.91[b]
dUrd	N_2O	0.34[b]		N_2O/O_2	0.46[b]
TMP	N_2O	0.1[b]	IMP	N_2O	0.77[b]
dThy-3′-P	N_2O	0.27[b]		N_2O/O_2	0.46[b]
dCCMP	N_2O	1.0[c]	dIMP	N_2O	0.35[b]
dAMP	N_2O	0.33[b]		N_2O/O_2	0.72[b]
	N_2O/O_2	0.64[b]			

[a] Dizdaroglu et al. (1976)
[b] Steenken and Schulte-Frohlinde, unpublished, reported in von Sonntag (1987a)
[c] Schuchmann et al. (1983)
[d] Ducolomb et al. (1971)
[e] Hems and Eidinoff (1958)

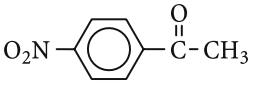
Typically, a mixture of such labile products are formed side by side, but more recently the precursor radicals of at least some of the labile products can be made quite specifically (e.g., the radical at *C*(1′), see above). In the future, this may al-

low an assignment of the various components to certain intermediates. Potential intermediates have been discussed in the preceding paragraphs.

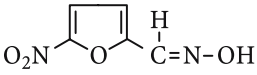
Further base release data are compiled in Table 10.31. There is a considerable spread in these values among the various nucleosides/nucleotides even under seemingly same experimental conditions. Part of this variation could be due to a change in the ratio of •OH attack at the base vs sugar moieties. The time elapsed between irradiation and work-up may also play a role (see Table 10.30). Compared to the 2′-deoxynucleosides, an additional site, *C*(2′), is likely to contribute. There is a dramatic difference between Ado and Ado-3′,5′-P. Phosphate release may here compete with base release. Further data are required to put this suggestion on a better footing.

10.9 Oxidation of Nucleobase/Sugar Radicals by Radiation Sensitizers

The oxidation of DNA radicals by hypoxic sensitizers, normally nitro compounds, has found considerable interest in the context of attempts to improve irradiation regimes in the radiotherapy of solid tumors (Chap. 12.11). Concomitantly, model studies have been undertaken in order to shed some light on potential mechanism of their reactions. *p*-Nitroacetophenone (PNAP) has often been used as a convenient model sensitizer.



PNAP



Nifuroxime

The radicals derived from 2-deoxyribose (ribose) upon •OH-attack yield 37% (23%) $PNAP^{\bullet -}$, and it has been concluded that ET only occurs from the *C*(1′) radicals (Michaels et al. 1976). With $^{\bullet}CH_2OH/CH_2O^{\bullet -}$ the rate of reaction with PNAP is only fast with the anion, $CH_2O^{\bullet -}$ (Adams and Willson 1973). Whether the radicals formed at the other sites in 2-deoxyribose and ribose give (slowly) rise to adducts cannot be deduced from the reported data. Yet, the fact that PNAP enhances the yield of free phosphate in the radiolysis of GMP (Greenstock et al. 1973a) is an indication that such adducts are likely to be formed, but the rate of reaction is very slow with α-phosphatoalkyl radicals ($< 5 \times 10^7$ dm^3 mol^{-1} s^{-1}; Schuchmann et al. 1995).

Practically no ET to PNAP occurs from the reducing Ura-6-yl Cyt-6-yl radicals, but the (protonated) Cyt electron adduct gives rise to $PNAP^{\bullet -}$ (Hissung and von Sonntag 1979). As mentioned above, the (protonated) electron adduct of dAdo is also rapidly oxidized by PNAP, but upon ET, dAdo is not reformed (Hissung et al. 1981a). Possibly, the resulting product retains the elements of water.

With a nitro compound that has a higher reduction potential than PNAP, e.g., nifuroxime, the rate of reaction with various radicals seems to be always near diffusion-controlled, and there is a competition between ET and adduct formation

(Greenstock and Dunlop 1973). The adducts are still radicals. As discussed in Chap. 6.3, they may decompose unimolecularly by releasing the sensitizer radical anion or undergo β-fragmentation yielding the nitroso compound and an oxyl radical (Nese et al. 1995). Although in its outcome the former reaction is equivalent to an ET, the branching (competition) occurs now at the level of the adduct.

Quinones are also strong oxidants, and their potential as radiation sensitizers has been investigated on the model level. Again, ET competes with addition (Simic and Hayon 1972, 1973; Hayon and Simic 1973). With carbon-centered radicals, a C-C bond is formed in the addition reaction, and subsequent reactions are different (von Sonntag et al. 2004) from the adducts formed with nitro compounds.

10.10 Irradiation in the Solid State

Experiments in the solid state, especially EPR studies, have supplemented our knowledge in the area of the free-radical chemistry of nucleobases and related compounds considerably. Here, only some salient points can be mentioned, and for more detailed information the reader is referred to the excellent reviews that have appeared on this topic (Wyard and Elliott 1973; Bernhard 1981; Hüttermann 1982, 1991; Close 1993; Sevilla and Becker 1994; Becker and Sevilla 1997) and to the book by Box (1977).

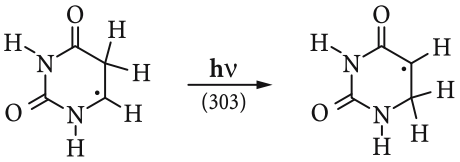
Mainly two types of experiments have been carried out, γ-irradiation in the solid state (mainly single-crystals) and in glassy matrixes at low temperatures. In addition, γ-irradiated solids may be dissolved in water containing spin traps, and the spin-trapped radicals identified by EPR afterwards (cf. Mossoba et al. 1981; Spalletta and Bernhard 1982; Zhang et al. 1983). While irradiation in a glassy matrix is very much related to the situation that prevails in aqueous solutions, that is, the radicals generated in the matrix react with the added substrates, irradiation in the solid state may cause radical formation via two different processes: ionization processes (formation of radical cation is and radical anions) and the decomposition of electronically excited states (which could also be formed upon recombination of a radical cation and an electron). It has often been tried to disentangle these two primary processes, but, of course, such assignments are not always straightforward. Sometimes, a confusing terminology persists which calls the electron-loss centers radical cations and the electron-gain centers radical anions, irrespective of their protonation state (i.e. charge sign).

Whenever H$^\bullet$-adducts are observed, they must not necessarily have H$^\bullet$ as precursor. Protonation of the radical anion must always be considered as an alternative/additional route. In crystals, the radical cations may serve as the proton source, while in frozen aqueous solutions the solvent will provide the proton.

Frozen solutions. In frozen aqueous solutions, the additive may not precipitate but accumulate in ice-free areas in a rather uncontrolled way. Upon irradiation, there are only a few radicals from the radiolysis of ice that reach the solute, and radical formation can occur by direct absorption of the energy of ionizing radia-

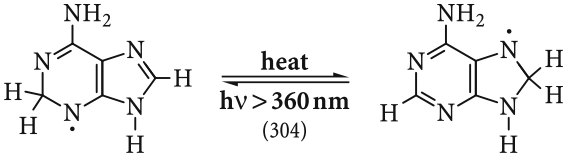
tion. In addition, erenkov radiation will photoexcite the solutes, and being aggregated they stand a chance of forming well-known photoproducts, e.g., in the case of Thd the cyclobutane dimers (Shaw et al. 1988). The long lifetimes of the radicals under these conditions make reactions possible that are not as readily observed in liquid water. For example, all conceivable 5′,6-5,6-dihydrocyclonucleosides were observed both for Thd and dUrd (Shaw and Cadet 1988), and the same cyclonucleoside as is formed with dUrd is also observed with dCyd (Shaw and Cadet 1996 or other Cyt deamination reactions; see above).

Single-crystals and powders. In Ura, the *N*(1)-centered radical is observed (Zehner et al. 1976). Based on our present knowledge, one may suggest that it arises most likely from the deprotonation of the radical cation. The radical anion is protonated at *O*(4). The *C*(5)-H•-adduct primarily formed is converted with light of $\lambda > 400$ nm into the thermodynamically more stable *C*(6)-H•-adduct [reaction (303)]. This is also observed with other pyrimidines (Flossmann et al. 1976).

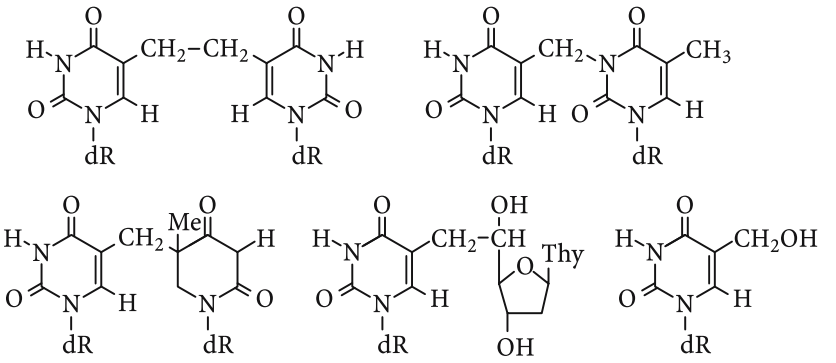


In 1MeUra, a predominating radical is the -CH_2• radical (Flossmann et al. 1973, 1975a,b). A -CH_2• radical (here, allylic) is also observed with Thy and 5MeCyt (Hüttermann 1970; Hüttermann et al. 1971; Dulcic and Herak 1973). Radical cations are likely precursors, while the precursor of the Thy *C*(6)-H•-adduct that is commonly observed (Henriksen and Snipes 1970) could be the Thy radical anion (Symons 1990; see above).

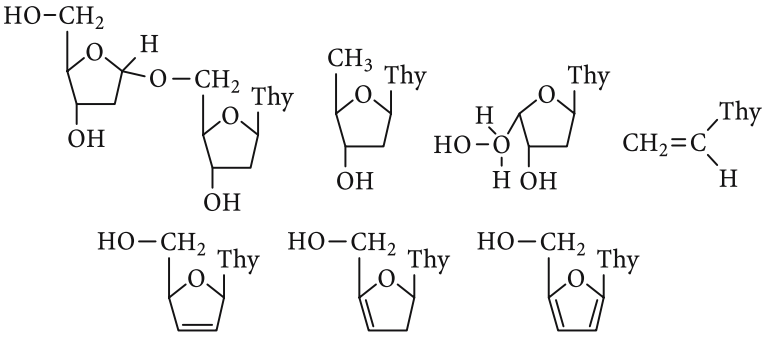
The ENDOR technique applied to an X-irradiated dAdo single-crystal allowed a complete analysis of the EPR parameters of the major radicals formed in that system (Nelson et al. 1998). The hole deprotonates at *N*(6) while the electron gain center protonates at *N*(3); •H-adducts are formed at *C*(2) and *C*(8). The thermodynamically more stable radical is the *C*(8) •H-adduct (see above), but in Ade light can convert the *C*(8) •H-adduct into the *C*(2) •H-adduct [Zehner et al. 1977; reaction (304)].



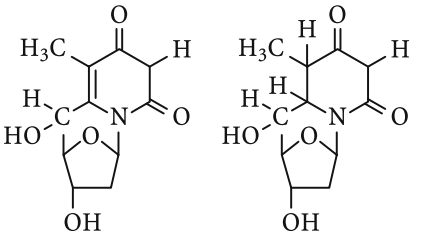
Photolysis of $G^{\cdot+}$ leads to the formation of sugar radicals (Adhikary et al. 2005). Product studies from solid state irradiation of Thd are also available (Gromova et al. 1999). As discussed above, the Thd radical cation is expected to deprotonate at *N*(3) and at methyl, and the ensuing structural elements indeed dominate the product spectrum parts of which is shown below.



The release of free base (see also Hoffman and Hüttermann 2000) and the formation of 2-dRL results from a damage of the sugar moiety, but there are a host of



further products altered at the sugar moiety.
The formation of the cyclonucleosides shown below (for their synthesis see Romieu et al. 1999b) shows that the addition of the *C*(5′) radical can also add to the *C*(5)-*C*(6) Thy double bond [the corresponding Cyd case has been discussed



above in reactions (195) and (196)].
In the irradiation of solid Thd the formation of •H-adducts is established (see above), and it is thus not surprising that H_2Thd (and also H_2Thy; secondary product?) are also among the products.

Similar experiments have been carried out with dGuo (Gromova et al. 1998). The free base, 2-dRL sugar lesions of analogous to those reported above (now

Table 10.32. Photosensitized oxidation of Thd by benzophenone and menadione. Product yields after 44% conversion of the educt. (Delatour et al. 1998)

	HMdUrd	FordUrd	Tg
Benzophenone	7.5%	20%	19%
Menadione	7.5%	16%	55%

with Gua as the base) have again been observed. The 8,5′-deoxy-cyclonucleoside, cG and the 5′-aldehyde (mechanistically interesting is the formation of two isomers; is an enol the intermediate?) are the other products that have been detected.

10.11 Photosensitization and Singlet Dioxygen Reactions

Photoexcited anthraquinone-2,6-disulfonate undergoes ET with Thy and its methyl derivatives, and the EPR results (Geimer et al. 1997; Geimer and Beckert 1998, 1999) have been discussed above.

Thy and Thd quench triplet menadione giving rise to the menadione radical anion (Wagner et al. 1990b). The corresponding Thy product has been considered to be the Thy radical cation, based on the rapid oxidation of TMPD. However, this intermediate has been shown to deprotonate rapidly ($pK_a = 3.2$; Geimer and Beckert 1998), and it is more likely that this rapid oxidation is given by the *N*-centered radical formed upon deprotonation of the radical cation. The reactions of menadione- and benzophenone-sensitized oxidation of Thd in the presence of O_2 lead mainly to HMdUrd, FordUrd and Tg (Decarroz et al. 1986; Delatour et al. 1998; Table 10.32). It has been suggested that the reaction mainly proceeds via an ET (formation of the Thd radical cation), but H-abstraction at methyl has not been excluded as an additional pathway. Qualitatively, these two sensitizers yield the same products, but interestingly, the material balance is much better in the case of menadione sensitization. The reason for this is not yet known.

The products that are formed upon photosensitization of dCyd by menadione were already given in Table 10.16 together with those formed upon $^{\bullet}OH$-attack. It has again been suggested that the precursor of these products is the dCyd radical cation formed by ET to excited menadione (Decarroz et al. 1987).

The riboflavin triplet reacts with dGMP acid by ET ($k = 6.6 \times 10^9$ dm^3 mol^{-1} s^{-1}), and evidence for the formation of the (deprotonated) Gua radical cation has been obtained by laser flash photolysis (Lu et al. 2000). The photosensitized reactions of dGuo by TRP is thought to follow two pathways, the formation of Z has been attributed to an ET reaction (Type I), and the reaction of singlet dioxygen [$O_2(^1\Delta_g)$; Type II] leads to 4-OH-8-oxo-G and 8-oxo-G (Ravanat et al. 1998). The effect of D_2O and azide on the 4-OH-8-oxo-G yields shows that this

Table 10.33. Photosensitization of dGuo by various sensitizers. Ratio of product distribution. (According to Ravanat et al. 1998)

Photosensitizer	(4-OH-8-oxo-G + 8-oxo-G)/Z
Zn-TRP	5.6
Methylene blue	3.6
TRP	2.3
Riboflavin	0.4

TRP = μ[meso-5,10,15,20-tetra(pyridyl)porphyrin]tetrakis[bis(bipyridine)chloride ruthenium(II)]

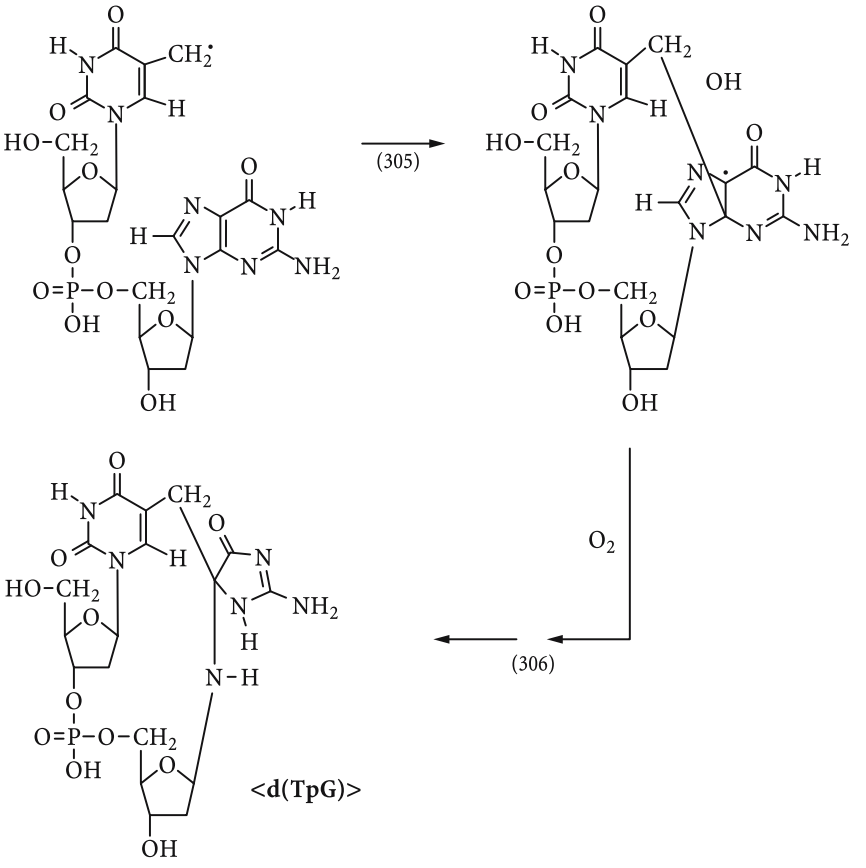
Table 10.34. Effect of photoexcited menadione, benzophenone and riboflavin on Thd, dGuo and d(TpG). Degradation (+ = observed, - = not observed) and specific products in the case of d(TpG). (Delatour et al. 1999)

	Menadione	Benzophenone	Riboflavin
Thd	+	+	–
dGuo	–	+	+
d(TpG)	+	+	+
T → HMdU/FordU	+	+	–
dG → dZ	–	+	+
<d(TpG)>	+	+	–

product results from the $O_2(^1\Delta_g)$ reaction (for the synthesis of oligonucleotides that contain this lesion see Romieu et al. 1999d). The products are very sensitive to further degradation, even at low conversions. In a mixture of dGuo and 8-oxo-G, the latter is practically fully degraded before dGuo starts to be consumed (Ravanat et al. 2003). This has been attributed to the rapid oxidation of 8-oxo-G by $G^{\bullet}$ ($k = 4.6 \times 10^8$ dm^3 mol^{-1} s^{-1}; Steenken et al. 2000). Moreover, the reaction of $O_2(^1\Delta_g)$ with 8-oxo-G readily yields as the major product 1-(2-deoxy-β-D-erythro-pentofuranosyl)cyanuric acid (Raoul and Cadet 1996), but Z is also among the products (Ravanat et al. 1998). In fact, methylene blue photooxidizes 8-oxo-G three times faster than dGuo (Buchko et al. 1995a). As a consequence, the ratio of the product yields, shown for various sensitizers in Table 10.33, may have a wide error bandwidth, but the data strongly indicate that sensitization by

riboflavin differs mechanistically from that of the other which mainly produce $O_2(^1\Delta_g)$.

These studies have been extended to the photooxidation of d(TpG) by riboflavin and methylene blue, and again Z and its precursor Iz have been characterized (Buchko et al. 1995b). When d(TpG) is photooxidized with benzophenone or menadione, an additional product, <d(TpG)>, is formed [reactions (305) and (305); Delatour et al. 1999]. It constitutes a tandem lesion.



The effects of the three sensitizers, menadione, benzophenone and riboflavin that have been investigated with respect to their reactivity towards Thd, dGuo and d(TpG) are compared in Table 10.34. Riboflavin is only capable of sensitizing dGuo and the Gua moiety of d(TpG), and thus does not give rise to <d(TpG)>. In contrast, menadione does not sensitize dGuo or the Gua moiety of d(TpG).

Mechanistically, it is likely that in the first step to <dTpG)> the allylic Thy radical is created. There are two possibilities: (a) ET from Thy to the sensitizer and deprotonation of $Thy^{\bullet+}$ or (b) H-abstraction of the allylic hydrogen by the sensitizer. The authors favor route (a). Yet $Thy^{\bullet+}$ should oxidize rapidly the neighboring Gua moiety. Moreover, it is difficult to see, why excited menadione

does not oxidize dGuo, although Gua is more easily oxidized than Thy. Another intriguing aspect is the report that the riboflavin triplet reacts rapidly with dUMP by ET (Lu et al. 2000), but no products that should be formed from this type of reaction are observed for Thd (Delatour et al. 1999) that should undergo that reaction as easily. For explaining the formation of the observed products, the allylic Thy radical has to add to *C*(4) of the neighboring Gua moiety [reaction (305)], and addition of O_2 to the Gua radical thus generated will convert in subsequent reaction this part of the molecule into Z [reaction (306)]. Reaction (306) is in competition with an O_2-addition to the allylic Thy radical that leads to the other observed product, the HMdU and FordU derivatives.

Photooxidation of the dGuo-5′-lysine ester leads to Z cross-linked to the lysine moiety (Morin and Cadet 1995), indicating that DNA-protein cross-links may also occur via such a process in case this amino acid happens to be in a proper position for the reaction to proceed.

Photooxidation of Ade by menadione results in a complex sequence of reactions to a formylation and acetylation of Ade at *N*(6) (Wang and Liu 2002). These groups stem from the sensitizer.

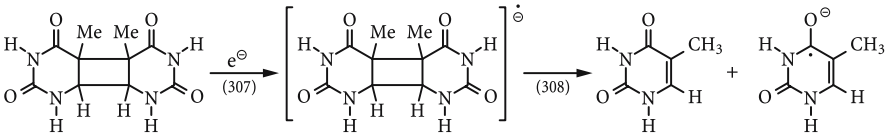
Photooxidation of purine nucleosides and also of Cyt by pyrimido[5,4-*g*]pteridine *N*-oxide under argon affords in high yields the 5′-*O*,8-cyclopurine nucleosides and 5′-*O*,6-cyclocytidne, whereby the *N*-oxide is reduced (Sako et al. 1986). Nucleobase radical cations are believed to be the intermediates in this surprising oxidation reaction.

10.12 Oxidation by Transition Metal Ions in their High Oxidation State

Transition metal ions in their high oxidation state have also been used to oxidize DNA components. These reactions may involve one-electron oxidation steps, i.e., free-radicals may also play a role. Oxoruthenium(IV) complexes oxidize GMP an order of magnitude faster (k = 6.1 - 15 $dm^3\ mol^{-1}\ s^{-1}$) at the base moiety than dCMP (0.24 - 0.47 $dm^3\ mol^{-1}\ s^{-1}$), where the sugar moiety is attacked (Farrer and Thorp 2000).

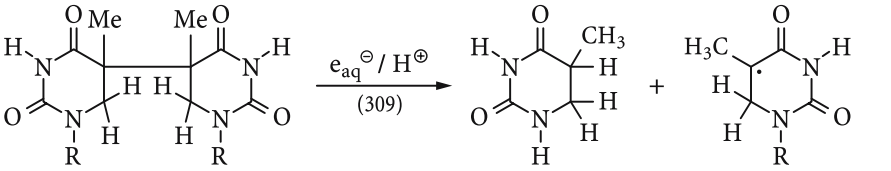
10.13 Free-Radical-Induced Transformations of Damaged Nucleobases

A number of studies are concerned with the free-radical reactions of typical nucleobase lesions. For example, the cyclobutane-type Thy dimer can be split by one-electron reduction [Heelis et al. 1992; reactions (307) and (308)], a process that is relevant to the repair of this typical UV-damage by the photoreactivating enzyme (photolyase, for a review see Carrell et al. 2001, for the energetics of the complex reaction sequence, see Popovic et al. 2002). At 77 K, the dimer radical anion is sufficiently long-lived to be detectable by EPR (Pezeshk et al. 1996).

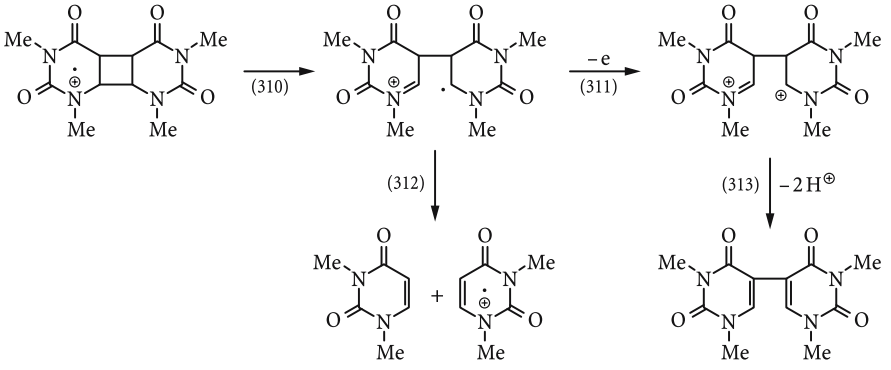


The dimer can be split by e_{aq}^- and by other reducing radicals such as $CO_2^{\bullet -}$ or $^{\bullet}C(CH_3)_2OH$, albeit with a much lower efficiency. The resulting Thy radical anion is also capable of transferring an electron to the Thy dimer, and this leads to a short chain reaction.

The *C*(5)-*C*(5′)-linked dihydrothymine dimers are also readily split by e_{aq}^- [reaction (309)] forming H_2Thy but also Thy in appreciable yields (Ito et al. 2000).



Reaction with $^{\bullet}OH$ also leads to the splitting of the dimer (~30% efficiency), and there is evidence that one-electron oxidants such as $SO_4^{\bullet -}$ may also induce the splitting of the dimer (Heelis et al. 1992). The $^{\bullet}NO_3$-radical-induced splitting of the tetramethyl-substituted Ura cyclobutane dimer has been investigated in acetonitrile (Krüger and Wille 2001). The $^{\bullet}NO_3$ radical has been generated photolytically from a Ce(VI) salt (Chap. 5.2). Under theses conditions, the 5-5′-linked intermediate is also trapped, possibly by a deprotonation or a Ce(IV)-mediated oxidation that competes with β-fragmentation [reactions (310)–(313)].

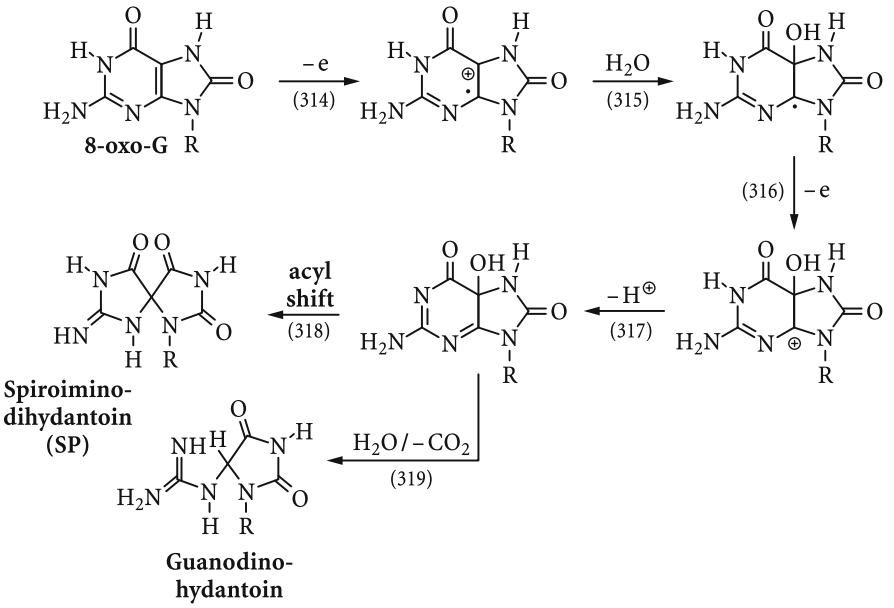


One-electron oxidation of the 5,5′-linked dihydrothymine dimer by $SO_4^{\bullet -}$, $N_3^{\bullet}$ or photoexcited anthraquinone-2-sulfonate also affords Thy together with H_2Thy (Ito et al. 1999).

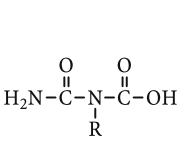
The reaction of $CO_2^{\bullet -}$ with Tg affords Thy, H_2Thy and 6OHH_2Thy (Nishimoto et al. 1985). The yield of the former two products is noticeably enhanced

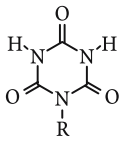
in the presence of Fe^{2+}. Photoreduction of Tg by aromatic amines leads such as TMPD, leads to Thy (72%) and $6OHH_2Thy$ (27%) (Ide et al. 1985).

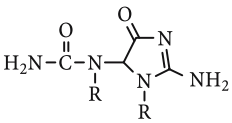
The reduction potential of 8-oxo-G is low [E^7 = 0.74 V/NHE (Steenken et al. 2000); +0.6 V (Berger et al. 1990)]. It is thus more easily oxidized than dGuo, and may act as a sink of oxidizing radicals (Doddridge et al. 1998), including $^{\bullet}NO_2$ ($k = 5.3 \times 10^6$ dm^3 mol^{-1} s^{-1}; Shafirovich et al. 2001). It is about 1000-fold more reactive towards peroxynitrite than dGuo (Burney et al. 1999; Niles et al. 1999; for a nitration product, see Niles et al. 2000; for a study in ODNs see Tretyakova et al. 1999; for the repair of such lesions see Duarte et al. 1999). With such a low reduction potential, it may even be oxidized by peroxyl radicals whose reduction potentials range between 0.77 and 1.6 V (Chap. 8.3). Upon its oxidation, spiroiminodihydantoin (Sp) is formed as the major product [reactions (314)–(318); Luo et al. 2000]. To a lesser extent some decarboxylation also occurs [reaction (319)]. This product has been characterized in some detail (Luo et al. 2001b).

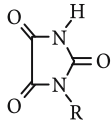


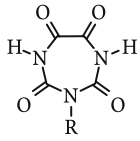
Sp is also a major (final) product in the oxidation of dGuo by triplet states (Luo et al. 2001a; Adam et al. 2002a,b; for an earlier study see Adam et al. 1996). Further reported oxidation products of 8-oxo-G are oxaluric acid (by $O_2(^1\Delta_g)$; Duarte et al. 2000), cyanuric acid, iminoallantoin, parabanic acid and 1,3,5-triazepane-2,4,6,7-tetrone (Hickerson et al. 1999). Oxaluric and parabanic acids may also be formed upon the reaction of the 8-oxo-G radical with $O_2^{\bullet-}$ (Misiaszek et al. 2005; Chap. 12.3).


Oxaluric acid


Cyanuric acid


Iminoallantoin


Parabanic acid


1,3,5-Triacepane-2,4,6,7-tetrone

$O_2(^1\Delta_g)$ not only oxidizes dGuo to 8-oxo-G, but the latter reacts even two orders of magnitude faster with this oxidant (Sheu and Foote 1995a), whereby the 4-hydroperoxide is formed via an unexpected rearrangement of the dioxetan intermediate (Sheu and Foote 1995b). The water-soluble endoperoxide derived from *N*,*N*′-di(2,3-diydroxypropyl)-1,4-naphthalenedipropanamide is a clean source of $O_2(^1\Delta_g)$, and can even prepared with an ^{18}O label. Using this approach, the oxygen label in the final products, Iz, Z, Sp and guanodinohydantoin was detected (Martinez et al. 2002).

The dCyd oxidation products 5-hydroxy-dCyd and 5-hydoxy-dUrd have low oxidation potentials (Wagner et al. 2004) and are hence likely to be oxidized further. For this reason the oxidation of 4-hydroxy-dUrd has been studied (Rivière et al. 2004). Using Br_2 or Na_2IrBr_6 but also menadione plus UV as oxidants, the main oxidation products were the isodialuric acid, dialuric acid and hydantoin derivatives.

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11.1 General Remarks

Polynucleotides have been widely studied as a model system for DNA. However, only the ribo-series is available at reasonable costs, and for this reason more detailed studies have been largely carried out with poly(U), poly(C), poly(A), and poly(G). In water, these polynucleotides remain largely single-stranded with the exception of poly(G) which has a tendency to aggregate, even above double-strandedness. This has made these polynucleotides, especially poly(U), the preferred tool to study the kinetics of radical-induced strand breakage, when it had been discovered that this process is connected with a change in conductivity and thus could be readily followed by pulse radiolysis (Bothe and Schulte-Frohlinde 1982; Chap. 13.3). Although these studies have yielded very important informations, the *ribo*-polynucleotides show some properties not given by DNA, not even by ssDNA. As a consequence, polydeoxyribonucleotides and smaller ODNs are finding increasing attention as DNA model systems. The OND nucleotide sequence can be designed to remain either ss or to form stable dsDNA sections with the advantage that they are small enough for a detailed analysis of the ongoing processes. These studies have become possible only during more recent years, when automated DNA synthesis became a well-established technique and these ODNs more reasonably priced. When appropriate, the results of the studies using dsODNs are discussed in Chapter 12.

In the present chapter, it will be shown that in the polynucleotides reactions take place that are not apparent on the nucleotide level (Chap. 10). The increasing number of reactions available to polymer-derived radicals (Chap 9) renders the free-radical chemistry of polynucleotides and ODNs more complex than that of the corresponding nucleotides. Nevertheless, our understanding of mechanistic details is not on a much lower level, because with polymers the pulse radiolysis technique (Chap. 13.3) provides additional information.

11.2 Hydroxyl-Radical-Induced Reactions

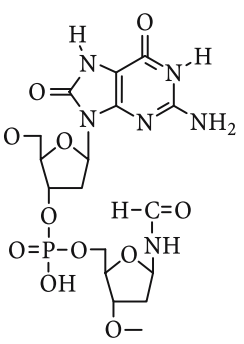
11.2.1 Small Oligonucleotides

The number of studies on ssODNs is rather low, but important information as to the formation of tandem lesions has been obtained with these systems.

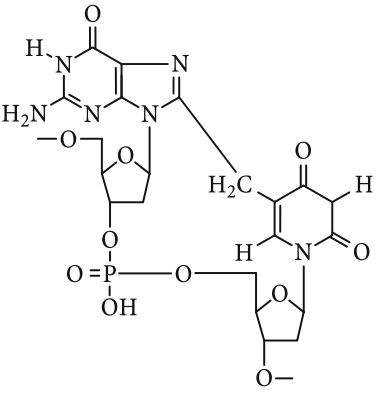
In the reaction of $^{\bullet}OH$ with d(TpApCpG) in oxygenated aqueous solution the Fo lesion, the Cyt-derived 1-carbamoyl-2-oxo-4,5-dihydroxyimidazolidine modification and 8-oxo-G were observed (Paul et al. 1988). In the presence of GSH and in the absence of O_2, the major detected modification is $5OHH_2Thy$ (Paul et al. 1990).

With d(ApCpGpT) X-irradiated in N_2-saturated solution the H_2Thy, $6OHH_2Thy$, Tg, 8-oxo-G and cA modifications were detected (Schroder et al. 1995). In the presence of O_2, the observed modifications were 8-oxo-G, 8-oxo-A,

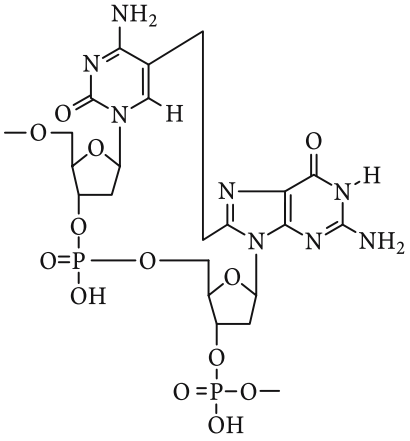
5-methyl-5-hydroxyhydantoin (from T) and 1-carbamoyl-2-oxo-4,5-dihydroxy-imidazolidine (from C). Moreover, the tandem lesion consisting of an 8-oxo-G next to a Fo (from T) lesion was also identified (for mechanistic aspects and importance of tandem lesion, see Chaps 10.6 and 12.5). Besides the 8-oxo-G/Fo lesion (in the presence of O_2), the tandem lesion, linking the methyl group of T to *C*(8) of a neighboring G, is observed with d(CpGpTpA) in the absence of O_2, and it has been noted that the tandem lesions were of greater importance than any of the simple lesions (Box et al. 1996; Budzinski et al. 1997). The scission products dCp, d(CpGp), dpA, d(pTpA) and d(GpTpA) were also detected.

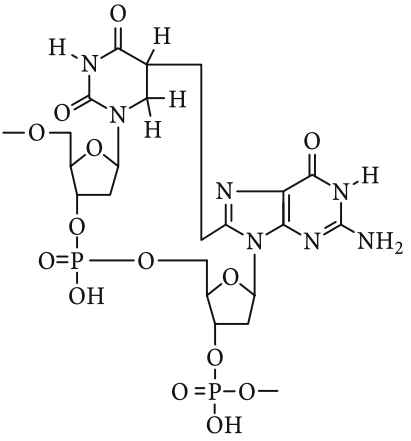


8-oxo-G/Fo



In an extension of this study, some further tandem lesions were detected in d(CpGpTpA) and d(CpApTpG) (Box et al. 1998). The cross-linked tandem base lesions shown below are only formed in the absence of O_2.





It is reasonable to assume that the H$^\bullet$- and $^\bullet$OH-adducts at *C*(5) of C bind to *C*(8) of the neighboring G, and the resulting adduct radical is oxidized in subsequent free-radical termination reactions. While the H$^\bullet$-adduct product (on the right)

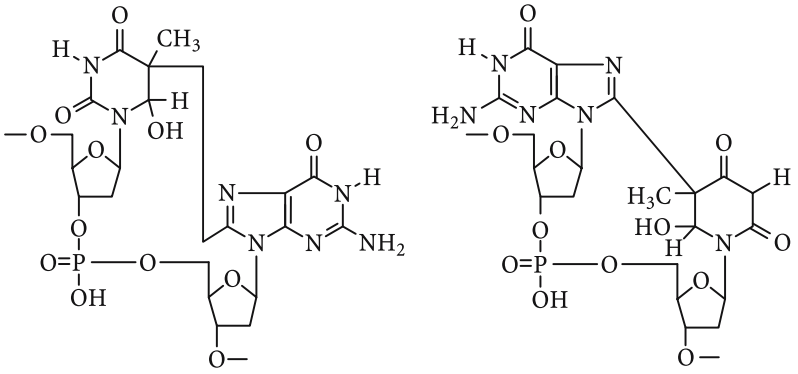
Table 11.1. Rate of oxidation of some oligonucleotides by $CO_3^{\bullet-}$ and $SO_4^{\bullet-}$. (Joffe et al. 2003b; Shafirovich et al. 2004)

Radical	Oligonucleotide	k/dm^3 mol^{-1} s^{-1}
$CO_3^{\bullet-}$	5′-dCCATC**G**CTACC	2.4 (± 0.3) × 10^7
$CO_3^{\bullet-}$	5′-dCCATC[**8-oxo-G**]CTACC	3.2 (± 0.4) × 10^8
$CO_3^{\bullet-}$	Duplex d(TATAAC**G**TTATA)	1.4 (± 0.2) × 10^{6a}
$CO_3^{\bullet-}$	Duplex d(AACGCGAATTCGCGTT)	1.9 (± 0.2) × 10^7
$CO_3^{\bullet-}$	5′-dCCATC[**Sp**]CTACC	5.8 (± 0.6) × 10^7
$SO_4^{\bullet-}$	5′-dCCATCGCTACC	3.2 (± 0.3) × 10^9

[a] At 15 °C

subsequently deaminates, the •OH-adduct product (on the left) must have lost water in an otherwise not yet observed 're-aromatization' reaction.

The same type of reaction is given by the •OH-adduct at *C*(6) of the T moiety.



The reaction not only proceeds in the 5′→3′ direction but also in the reverse direction as shown by the products depicted above.

ODNs have also been used for the study of the reactive intermediates that play a role in peroxynitrite reactions such as $CO_3^{\bullet-}$ and $^{\bullet}NO_2$ (Chap. 2.4). The $CO_3^{\bullet-}$ radical reacts quite fast with G-containing ODNs (Table 11.1).

In these ODNs, G is oxidized to 8-oxo-G. This product is much more easily oxidized by $CO_3^{\bullet-}$ and gives rise to the Sp lesion which is also readily oxidized (for mechanistic details see Chap. 10.14).

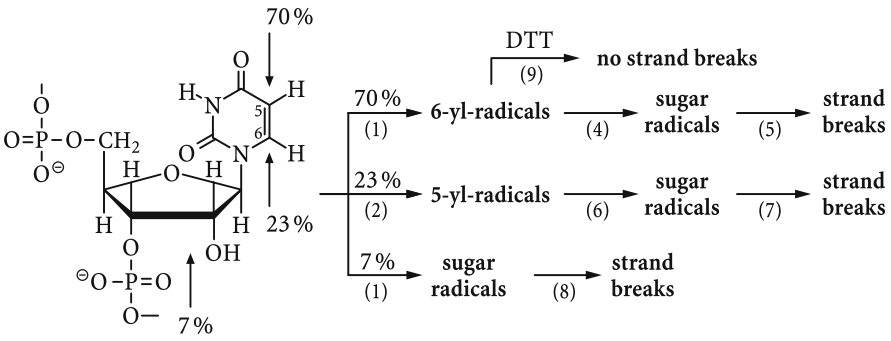
Table 11.2. Radiolysis of N_2O- and N_2O/O_2-saturated solutions of polynucleotides, polydeoxyribonucleotides and ssDNA. *G* values of counter-ion release and strand breakage (unit: 10^{-7} mol J^{-1}). (Lemaire et al. 1984; Adinarayana et al. 1988)

Substrate	G (counter-ion release)	G (strand breakage)
N_2O-saturated		
Poly(U)	20	2.4
Poly(A)	4.4	0.44
Poly(C)	18	2.2
Poly(G)	0.4	<0.2
Poly(dA)	20	2.3
Poly(dC)	12.3	1.4
Poly(dT)	5.0	0.57
Poly(dU)	4.1	0.46
Poly(dG)	0.75	0.09
ssDNA	7.3	0.83
N_2O/O_2-saturated		
Poly(U)	23	2.6
Poly(A)	8.5	1.04
Poly(C)	10.5	1.25
Poly(G)	≈ 0	<0.2
ssDNA	4.6	0.62

The $^{\bullet}NO_2$ radical does not react with any of the nucleobases, but when $G^{\bullet}$ is formed by one-electron oxidation, e.g., by $CO_3^{\bullet-}$, in the presence of $^{\bullet}NO_2$, recombination occurs thereby forming 8-nitroguanine (Joffe et al. 2003a).

11.2.2 Poly(U)

Poly(U) is predominantly attacked by $^{\bullet}OH$ at the base moiety (93%; 70% add to the *C*(5) position and 23% to the *C*(6) position) and only a small fraction of $^{\bullet}OH$ (7%) abstracts an H atom from the sugar moiety [reactions (1)-(3)] (Deeble et al. 1986).



Although strand breakage must have sugar radicals as the precursor, strand breakage is a much more important process than can be accounted for by the primary $^{\bullet}$OH-attack at the sugar moiety (G(strand breakage) = 2.4×10^{-7} mol J^{-1}; Table 11.2).

It is hence obvious that a radical transfer must occur from the base to the sugar moiety [reactions (4)–(7)]. In agreement with this, strand breakage and the decay of the absorption of the base radicals follow the same kinetics (Jones and O'Neill 1991). This radical transfer is also evident from the high yields of unaltered Ura (G(Ura) ≈ 3.0×10^{-7} mol J^{-1}; Deeble and von Sonntag 1984; Deeble et al. 1986; Hildenbrand et al. 1993). There must be more than one precursor. This is evident from the kinetics of base release: only 20% are released during (or immediately after) irradiation, while 80% are liberated at a much later stage, 50% in a fast and 30% in a slow process. The fast and the slow processes are only observable at elevated temperatures (Table 11.3).

In basic solution, the observed rate of strand breakage after $^{\bullet}$OH-attack is low (0.4 s^{-1}) and independent of pH, but increases by about three orders of magnitude in acid solution (Bothe and Schulte-Frohlinde 1982) (in neutral solution, a similar value, 3.7 s^{-1}, has been obtained by time-resolved light-scattering; Jones and O'Neill 1991). Thus, there is a spontaneous and a H^+-catalyzed reaction.

The major part (70%) of the $^{\bullet}$OH-induced strand breakage is prevented by thiols such as DTT (Lemaire et al. 1987). This has been interpreted as being due to a reduction of the C(5)-$^{\bullet}$OH-adduct [reaction (9); $k = 1.5 \times 10^6$ dm^3 mol^{-1} s^{-1}]. The (oxidizing) C(6)-$^{\bullet}$OH-adduct may not react with DTT (for the paradoxical behavior of thiols in their reactions with reducing and oxidizing radicals see Chaps. 6.5 and 7.4) and continue to contribute to strand breakage via a radical transfer to the sugar moiety. The rate of reaction of thiols with the negatively charged polyanion poly(U) most strongly depends on the charge of the thiol (Table 11.4).

TNM nearly completely suppresses strand breakage (G(strand breaks) = 0.2×10^{-7} mol J^{-1} remaining; Lemaire et al. 1987) and Ura release (Deeble and von Sonntag 1984). TNM oxidizes rapidly the C(5)-$^{\bullet}$OH-adduct, but may (on a much longer time scale) also form an adduct with the C(6)-$^{\bullet}$OH-adduct. This would prevent the radical transfer reactions from the base $^{\bullet}$OH-adducts to the sugar moiety leaving $^{\bullet}$OH-reactions with the sugar moiety as the only source of strand breakage. This low yield of directly induced strand breaks (Washino et al. 1983)

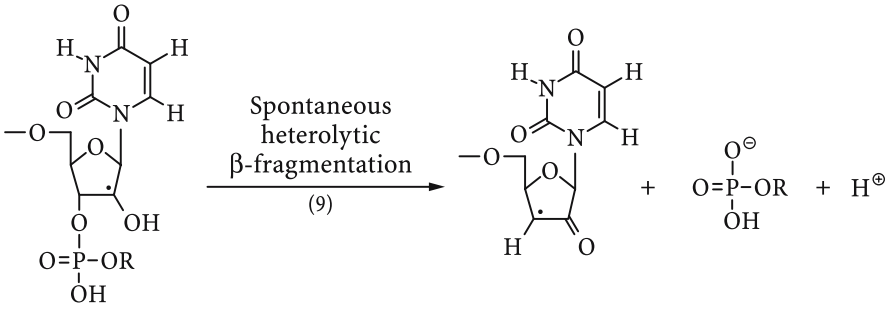
Table 11.3. Kinetics and yields (G; unit: 10^{-7} mol J^{-1}) of Ura release in the γ-radiolysis of poly(U) in N_2O-saturated aqueous solution. (Deeble et al. 1986)

	Immediate	Fast	Slow
G(Ura)	0.57	1.5	1.0
Percentage of total G(Ura)	19	48	33
$t_{½}$ at 0 °C	< 4 min		
$t_{½}$ at 40 °C		2.3 h	58 h
$t_{½}$ at 50 °C		1.23 h	26 h
$t_{½}$ at 60 °C		0.83 h	10.7 h
$t_{½}$ at 70 °C		0.32 h	3.7 h
E_a/kJ mol^{-1}		68	89
S_a/J mol^{-1} K^{-1}		–116	–75
A/s^{-1}		1.5×10^7	1.9×10^9
$t_{½}$ at 20 °C (extrapolated)		18 h	730 h

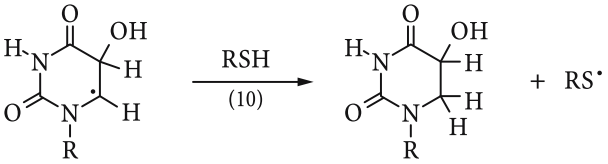
Table 11.4. Rate of repair of poly(U)-derived radicals by thiols at pH 7 and 4 (in parentheses). (Fahey et al. 1991)

Thiol	Net charge	k/dm^3 mol^{-1} s^{-1}	Relative rate
Glutathione (GSH)	–1	< 500	< 0.003
2-Mercaptoethanesulphonic acid	–1	1.5×10^3	0.009
2-Mercaptoethanol	0	1.8×10^5 (6.1×10^5)	1.0 (1.0)
Cysteine	0	2.0×10^5	1.1
Cysteamine	+1	4.1×10^7 (2.2×10^8)	230 (360)
WR-1065	+2	(4.6×10^8)	(760)

may be connected with the very fast component of strand breakage. The precursor radical could be the *C*(2′) radical which would yield a strand break within less than a few microseconds [reaction (9); Chap. 6.9)].



For a mechanistic interpretation several observations have to be taken into account: there is a considerable chromophore loss (G(chromophore loss) = 5.1 × 10^{-7} mol J^{-1} in N_2O-saturated solution; Deeble and von Sonntag 1984). The yield of the (acid-labile hydrate) 6OHH_2Ura must be low, because the Ura chromophore is not restituted upon heating (Deeble and von Sonntag 1984), while that of the acid-stable hydrate is also low (G(5OHH_2Ura) = 0.4 × 10^{-7} mol J^{-1}), and the addition of thiols increases its yield [reaction (10)], but not beyond 1.1 × 10^{-7} mol J^{-1} (Grachev et al. 1995, 1997).



It has been suggested (Hildenbrand and Schulte-Frohlinde 1989) that the H^+-catalysis in the rate of strand breakage may be due to the formation of a radical cation and subsequent rapid transformation into a sugar radical at *C*(2′) as discussed for the Cyd plus $SO_4^{\bullet -}$ reaction in Chapter 10.2 (cf. the rapid strand breakage observed after photoionization, see below). Indeed, at pH 4, the expected radical has been observed by EPR. This mechanism accounts for the close to equivalent yields of released Ura and strand breaks, but is somewhat in need to explain the observed substantial chromophore loss [and does not account for the low G(5-hydroxy-5,6-dihydrouracil)]. Yet it is recalled that the fate of the broken poly(U) strand which still carries the radical site remains unresolved. Apparently, they do not recombine efficiently (this would reduce the yield of strand breakage as measured by LALLS). In principle, they could add to a base within the broken strand thereby forming a small loop with the consequence of chromophore loss. This process could be slow (Chap. 10.5) and hence might have escaped being noticed in the pulse radiolysis experiments (Jones and O'Neill 1991). It does, however, require that in the final radical recombination reactions dimerization is negligible compared to disproportionation. This is usually not

Table 11.5. γ-Radiolysis of poly(U) in N_2O/O_2-saturated aqueous solution. Products and their *G* values (unit: 10^{-7} mol J^{-1}). (Deeble and von Sonntag 1986)

Immediate Ura release	1.6 ± 0.1
Total Ura release (95°C, 1 h)	5.5 ± 0.3
Carbon dioxide	2.8 ± 0.3
Total osazone forming compounds	2.8 ± 0.3
Glycoladehyde osazone	0.8 ± 0.2
Organic hydroperoxide(s)	7.1 ± 0.7
Hydrogen peroxide	1.8 ± 0.2
Chromophore loss	8 –11 (13)[a]
Dioxygen uptake	19 ± 3[a]

[a] From Isildar et al. (1982)

Table 11.6. *G*(free phosphomonoester groups) (unit: 10^{-7} mol J^{-1}) in the γ-radiolysis of polynucleotides in N_2O- and N_2O/O_2-saturated aqueous solution. (Murthy et al. 1988)

Polynucleotide	Additional treatment	N_2O	N_2O/O_2
Poly(U)	None	4.7	4.6
	Heated	6.1	7.5
	Borohydride	4.4	2.9
	Borohydride/heated	5.9	6.0
Poly(C)	None	1.7	2.1
	Heated	2.4	5.1
	Borohydride	1.5	1.1
	Borohydride/heated	2.3	2.8
Poly(A)	None	n.d.	n.d
	Heated	n.d.	0.5
Poly(G)	None	n.d.	≤0.4
	Heated	n.d.	0.5

n.d., Not determined

the case, but there are other polymers such as poly(vinyl alcohol) where cross-linking plays a very minor role (Chap. 9). Moreover, dimeric base products have been observed in poly(dT) after •OH-attack (Karam et al. 1986, 1988), similar to what had been observed with much smaller subunits (TpTpT and dCPdC; Dizdaroglu and Simic 1985).

In poly(U), H• also adds predominantly to the base moiety (60% at *C*(5), 40% at *C*(6); Das et al. 1985). It does not undergo any significant H-abstraction from the sugar moiety (Deeble, von Sonntag 1984), and yet it induces strand breakage (Bothe and Selbach 1985) and uracil release, the latter with 28% efficiency (Deeble and von Sonntag 1984). The Ura radical anion formed in the reaction of e_{aq}^- with poly(U) [known to become eventually protonated at *C*(6) thereby yielding the *C*(5)-H•-adduct, Chap. 10.4] does not give rise to Ura release to any significant extent (3%; Deeble and von Sonntag 1984). From this, it is evident that the radical transfer from the base to the sugar moiety is mainly induced by the *C*(6)-H•-adduct, and it is thus concluded that this must also hold for the induction of strand breaks. The rate of strand breakage is considerably slower than that induced by •OH, and also shows some, albeit much less pronounced pH dependence (1.5 s^{-1} at pH 4-5 and 2.5 s^{-1} at pH 3; Bothe and Selbach 1985). Suppression of strand breakage by DTT has been shown to occur at the stage of the base radicals ($k = 5 \times 10^6$ dm^3 mol^{-1} s^{-1}). The mechanism of strand breakage induced by H• must hence be considerably different from that by •OH and is not yet understood at present.

In the presence of O_2, the poly(U)-•OH-adducts are scavenged by O_2 ($k = 4.8 \times 10^8$ dm^3 mol^{-1} s^{-1}; Michaels and Hunt 1977b), and the subsequent chemistry is governed by the ensuing peroxyl radicals (Deeble and von Sonntag 1986). Apparently, the base peroxyl radicals can also abstract an H-atom from the sugar moiety (for the kinetics see Bothe et al. 1986). This leads to the formation of hydroperoxides (Table 11.5) and an increased O_2-uptake (Isildar et al. 1982). Acidic material is also formed whose yield increases upon heating (Schulte-Frohlinde and Bothe 1984).

H-abstraction must occur from a distant site. Otherwise, unaltered Ura would not be released. Its immediate yield is not very high, but increases with heating. This could be attributed to unstable precursors as has been discussed for deoxygenated solutions, but under the conditions of heating the hydroperoxides are unstable, and this may, in addition, lead to further free radicals, including •OH, thereby increasing the yields. A considerable doubling of free phosphomonoester groups has also been observed upon heating (Murthy et al. 1988), but reduction with borohydride which destroys the hydroperoxides cannot prevent this effect (Table 11.6). Thus, besides labile structures that yield additional free bases, such damaged sugars must give rise to additional free phosphomonoester functions. Borohydride reduction (without further heating) may prevent the formation of additional phosphomonoester functions, and under such conditions their yields compares reasonably well with the yield of strand breakage (Table 11.2).

The reduction of the poly(U)- and poly(A)-derived peroxyl radicals by thiols had been believed to be rather fast, ~10^5 dm^3 mol^{-1} s^{-1} (Schulte-Frohlinde et al. 1986a), but it has been more recently shown by the same group (Hildenbrand and Schulte-Frohlinde 1997) that the rate constants must be rather low ($\leq 2 \times$

10^2 dm^3 mol^{-1} s^{-1}), in agreement what has been found for the reaction of $HO_2^{\bullet}$ with DTT (120 dm^3 mol^{-1} s^{-1}; Lal et al. 1997).

Strand breakage is reported to follow largely first-order kinetics ($t_{½} \approx 3$ s; i.e. similar to that observed in the absence of O_2; monitored by conductance changes in a pulse radiolysis experiment), but at least for high-molecular-weight poly(U) there is a dose-per-pulse related component which points a second-order contribution (Schulte-Frohlinde et al. 1986b). Although there is some influence of pH on the observed rate, it is not as pronounced as in the absence of O_2. A pH-independent first-order process would be compatible with an H-abstraction from the *C*(2′)-position with subsequent very rapid (i.e. O_2-independent) strand breakage [reaction (9)]. The same process has also been studied by time-resolved laser light-scattering, where two contributions were noticed, a fast one ($t_{½} \leq 50$ µs, 20%) and a slow one ($k \approx 1.6$ s^{-1}, 70%, with a even slower component of about 10%; Jones and O'Neill 1990). In agreement with the conductivity measurements, the kinetics of the slow process are of first order (no effect of dose rate).

11.2.3 Poly(C)

Poly(C) behaves similarly to poly(U) in its $^{\bullet}$OH-induced reactions as indicated by the high strand breakage yields (Müller 1983) and concomitant release of monophosphate residues (Table 11.6). Moreover, spontaneous $G(\text{Cyt}) = 1.2 \times 10^{-7}$ and 2.3×10^{-7} mol J^{-1} after heating for 2 h at 95 °C are found in N_2O-saturated solutions (Hildenbrand et al. 1993). The kinetics of strand breakage (7.9 s^{-1}; Jones and O'Neill 1991) and also the yields are also very similar to those obtained with poly(U); the reaction is, however, somewhat faster. Mechanistically, the reactions are considered to be analogous to those observed with poly(U) (see above). In agreement with this, oxidation of the cytosine-$^{\bullet}$OH-adducts by 1,4-benzoquinone leads to a reduction of strand breakage by ~60% (Bamatraf et al. 1998). Interestingly, the nitroxyl radical formed upon the addition of nitroarenes to the reducing *C*(5)-$^{\bullet}$OH-adducts also gives rise to strand breakage (Bamatraf et al. 1998), and it has been concluded that these radicals can also abstract an H-atom from the sugar moiety. In the presence of O_2, the kinetics of strand breakage resemble those of poly(U) (Jones and O'Neill 1990).

11.2.4 Poly(A)

In poly(A), the strand breakage yield is only a quarter of that observed with poly(U) or poly(C) (Table 11.2). Pulse radiolysis experiments using laser light-scattering for detection revealed two processes with $t_{½}$ ~120 µs and ~500 ms (Washino and Schnabel 1982; Washino et al. 1984). The slower one was quenched by cysteamine, wherefrom the rate of strand break has been calculated at 1.7 s^{-1} and that of the reaction of cysteamine with the precursor radicals at 3.4×10^6 dm^3 mol^{-1} s^{-1}. The low yields of strand breaks is also reflected in the comparatively low yields of base release $G(\text{Ade})_{\text{immediate}} = 0.55 \times 10^{-7}$ mol J^{-1} (Hildenbrand et al. 1993), 0.28×10^{-7} mol J^{-1} (Fuciarelli et al. 1987), $G(\text{Ade})_{\text{after heating}} = 1.0 \times 10^{-7}$ mol

J^{-1} (Hildenbrand et al. 1993). It is interesting, that the Ade yield after heating exceeds that of strand breakage. Only the slow and minor strand-breaking process that is suppressed by cysteamine may be due to a radical transfer from the base to the sugar moiety.

The spectral changes observed by pulse radiolysis have been attributed to unimolecular transformation reactions and the suppression of these reactions by cysteamine has been studied (Hankiewicz et al. 1992; Hankiewicz 1996, 1998). Among the products the formation of 8-oxo-A, FAPY-A (Alexander et al. 1987) and cA were noticed, whereby at pH 7 the *R* isomer dominates over the *S* isomer by a factor of 2.5 (G(total) = 0.09×10^{-7} mol J^{-1}; Fuciarelli et al. 1986). This low yield is in contrast to a value of 1.4×10^{-7} mol J^{-1} obtained by an immunochemical assay (Fuciarelli et al. 1985).

In the presence of O_2, the $^{\bullet}OH$-induced absorption is decreased, but at different wavelength at different rates, and rate constants ranging from 3×10^8 dm^3 mol^{-1} s^{-1} to 2.9×10^9 dm^3 mol^{-1} s^{-1} have been obtained (Hankiewicz 1995). A low rate constant (5×10^7 dm^3 mol^{-1} s^{-1}) for O_2-addition has also been reported for poly(A+U) (Michaels and Hunt 1977a), but further details are not yet known. Apparently, low H_2O_2 concentrations also had an effect, and a rate constant as high as 10^{10} dm^3 mol^{-1} s^{-1} has been given (Hankiewicz 1995). This is an exceptionally high rate constant for a radical/H_2O_2 reaction (Chap. 6.7), and these results require further confirmation.

At low pH, poly(A) turns double-stranded, and at relatively high ionic strength and low doses per pulse cross-linking is observed, while DSBs are formed at low ionic strength and high doses per pulse (Denk et al. 1983). Similar results have been obtained with poly(A+U). With poly(A), also a dramatic effect of Mg^{2+} on these reactions has been noted.

It is concluded that despite all this effort the free-radical chemistry of poly(A) is still poorly understood.

11.3 Sulfate-Radical-Induced Reactions

The reaction of $SO_4^{\bullet-}$ with poly(U) and poly(C) in deoxygenated solutions results in the formation of strand breaks with efficiencies of 57 and 23%, respectively (Wolf et al. 1993). Most strand breaks are formed within 70 μs (the rise time of the time-resolved light-scattering detection system), that is, several orders of magnitude faster than the rate of strand breakage induced by $^{\bullet}OH$. In the presence of O_2, strand breakage is suppressed. This is in contrast to photoionization, where also rapid strand breakage (here induced by the Ura radical cation) is observed but not suppressed by O_2 (Görner et al. 1992). These observations led to the conclusion that an $SO_4^{\bullet-}$-adduct must be responsible for the induction of strand breakage. From this, it also follows that upon the reaction of poly(U) and poly(C) with $SO_4^{\bullet-}$ the Ura/Cyt radical cations are not formed. Instead, it has been proposed that nucleophilic attack by the *C*(2′)OH group eliminates sulfate from the $SO_4^{\bullet-}$-adducts (Catterall et al. 1992; Aravindakumar et al. 2003). Mechanistically, strand breakage in poly(C) and poly(U) is related to base release in

the corresponding nucleosides, Cyd and Urd, i.e. the C(2′) radical is the common precursor for both processes (for a detailed discussion that is not repeated here, see Chap. 10.2). Apparently, only a fraction of $SO_4^{\bullet -}$ induces strand breakage, and there must be competing processes such as a nucleophilic attack by *C*(2′)OH and by water.

Poly(A) and poly(G) react with $SO_4^{\bullet -}$ by ET (Wolf et al. 1993). In poly(A) this reaction does not give rise to noticeable strand breakage (3%). The decay kinetics of the poly(A)-derived radicals have been followed by EPR, and it has been shown that it is biphasic; 60% decay at a rate of 2 s^{-1} and 40% at 50 s^{-1} (Schulte-Frohlinde and Hildenbrand 1989).

11.4 Photoionization

Photoionization has been widely used to produce radical cations to study the direct effect of ionizing radiation on DNA. In the case of 248 nm excitation of polynucleotides (and ssDNA), photoionization is largely a biphotonic process, although with poly(A), poly(C) and ssDNA there may be a small monophotonic contribution (quantum yield, $\Phi_{PI} < 10^{-3}$; Wala et al. 1990). At 193 nm, photoionization is a monophotonic process except for poly(U), where it is still biphotonic (Table 11.7). This excludes a major monophotonic contribution of the phosphate group to the ionization events at this wavelength ($\varepsilon \times \Phi_{PI}$; typical values for alkyl phosphates: ε(193 nm) ≤ 180 $dm^3\ mol^{-1}\ cm^{-1}$; $\Phi_{PI} \approx 0.5$; cf. Steenken and Goldbergerova 1998).

Photoionization at 193 nm in oxygenated solution of poly(C) causes strand breakage with high efficiency, half of which occurs at times < 4 ms, the other half with a half-life of 7 ms (Melvin et al. 1996; Table 11.8). This kinetic behavior is very different from what is seen after $^{\bullet}$OH-attack and points to the direct involvement of the Cyt radical cation. In poly(U), the (biphotonic) photoionization shows similar results (Table 11.8). With poly(A), the formation of strand breaks is 20-times less efficient as compared to poly(C) (Table 11.8), and this is in agreement with the above conclusion that the $A^{\bullet +}$ or $A^{\bullet}$ do not cause strand breakage to any major extent.

In poly(U), the slow component shows all the kinetic properties of strand break formation by the $^{\bullet}$OH-adducts (Schulte-Frohlinde et al. 1985). Thus, it is reasonable to assume that the fast component has been induced by the rapid transformation of the Ura radical cation into the *C*(2′) radical (for a mechanistic discussion of the analogous Cyd system see Aravindakumar et al. 2003 and Chap. 10.2). The branching (60% fast to 40% slow) may be due to a competition of *C*(2′)OH and water for the uracil radical cation.

In the 193-nm photolysis of poly(U), only low yields of base release compared to photoionization have been observed (Gurzadyan and Görner 1994). Only the prompt base release has been determined, and the question must be posed whether these values would also increase substantially upon heating as has been observed after $^{\bullet}$OH-attack (see above).

Table 11.7. Quantum yields of photoionization (Φ_{PI}) of polynucleotides and DNA by 193 nm laser excitation in aqueous solution; estimated error 30%. (Candeias et al. 1992)

Substrate	Φ_{PI}
Poly(G)	0.044
Poly(A)	0.034
Poly(C)	0.029
Poly(U)	Biphotonic (0.032 at 22 mJ/pulse)
ssDNA	0.043
dsDNA	0.036

Table 11.8. Laser flash photolysis at 193 nm of various ss polynucleotides and DNA samples with different base compositions (origin: ML = *M. lysodeikticus*, CT = calf thymus, CP = *C. perfringens*). Contribution of the fast component and the half-life of the slower component of the TRLS signal and the ratio of the quantum yield of single-strand breakage and photoionization. (Melvin et al. 1996)

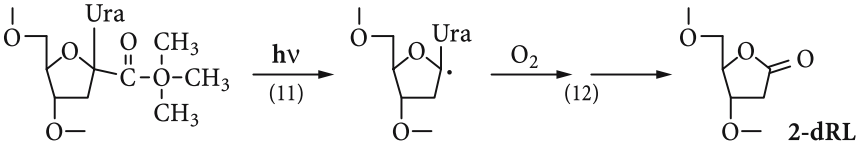
Polynucleotide	Fast component/%	$t_{½}$ (TRLS)/ms	$t_{½}$ (Optical)/ms	Φ_{SSB}/Φ_e
Poly(C)	50	6–7	7.0	0.32
Poly(U)	60	40	45	
Poly(A)	> 90			0.015
DNA ML (72% G:C)	< 10		40	
DNA CT (43% G:C)	<10	46	40	0.011
DNA CP (31% G:C)	<10	40		

11.5 Specifically Generated Radicals

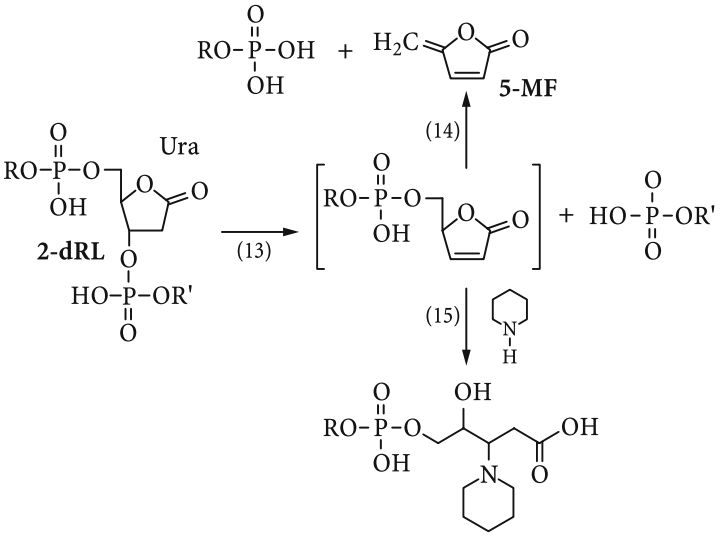
Introducing a photolabile substituent at *C*(5) of the H_2Thy moiety of oligo-dT allowed to generate specifically the Thy-5-yl radical (Barvian and Greenberg 1992). In the absence of O_2, no stand breakage was observed in agreement with the poor H-abstracting power of this radical noticed before (Barvian and Greenberg 1995b). In the presence of O_2, however, substantial strand breakage occurs (Barvian and Greenberg 1995a). Strand breakage is observed in the 5′-direction

up to three nucleotides away from the position of the Thy-5-peroxyl radical (Greenberg et al. 1997). With a G present at the 5′-nucleotide (followed by C- and T-containing nucleotides), strand breaks and alkali-labile sites occur mainly at G sites, five times greater than at the following C sites.

Introducing in the 5′-position a T-containing nucleotide labeled with deuterium at either *C*(1′), *C*(2′) or *C*(4′) showed only a marked kinetic isotope effect (KIE = 3.9) when labeled at *C*(1′). It has been concluded that the observed effects must be due to an H-abstraction of the Thy-5-peroxyl radical from the neighboring *C*(1′) position. A kinetic isotope effect is only observed when there is a competing reaction. This competing reaction has not yet been identified. However, some information has been obtained by generating specifically the *C*(1′) radical within a short ODN [reactions (11) and (12); Hwang et al. 1999].

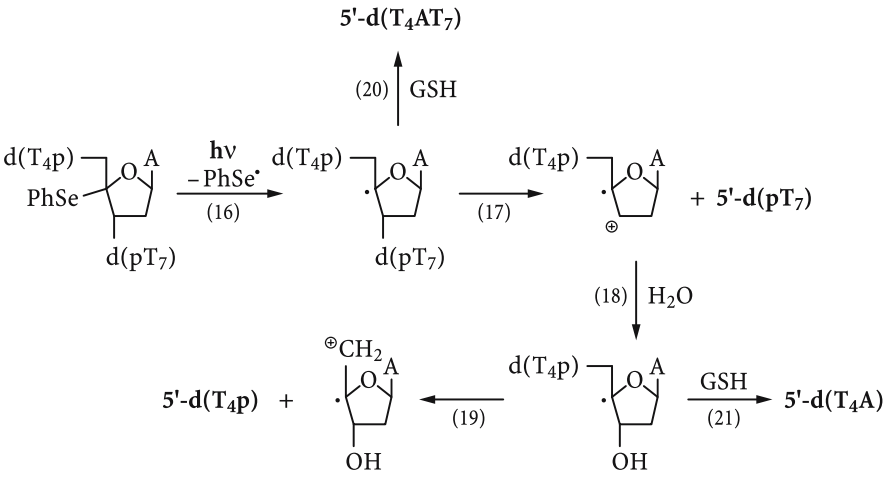


The 2-dRL lesion and the 3′-fragment resulting from reaction (13) were identified by mass spectrometry. The bona fide intermediate (in brackets) expected to be formed upon treatment with a base such as piperidine was not observed, but the β,δ-elimination product [reaction (14)] and an adduct of the amine [reaction (15)] were identified.

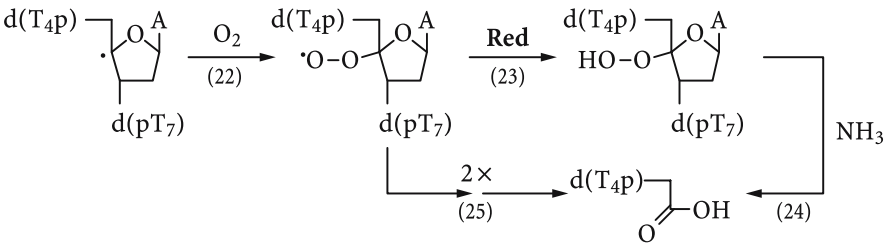


For studying mechanistic aspects of DNA strand breakage starting from the *C*(4′) radical, ODNs substituted by a phenylselenide group at *C*(4′) were photo-

lyzed. The products resulting in the absence of any additive were the two adjacent oligonucleotides [reactions (16)–(19); Giese et al. 1995].



In the presence of GSH, 5′-d(T_4AT_7) and 5′-d(T_4A) are formed [reactions (20) and (21)]. In the presence of O_2, the primary radical is trapped by O_2. In a subsequent step, the *C*(4′) peroxyl radical is reduced to the corresponding hydroperoxide (the source of the reduction equivalent is as yet unknown; potentially $O_2^{\bullet -}$ generated in side reactions), and treatment with NH_3 increases the yield of the glycolate which is also formed upon the bimolecular decay of the peroxyl radial [reactions (23)–(25)].



11.6. 5-Bromouracil-Substituted ssDNA Fragments

The photolysis of 5BrUra-substituted DNA has been widely used as tool to study certain aspects of DNA free-radical chemistry. The basic reactions that occur on the nucleobase to nucleotide level are discussed in Chapter 10.7. Some of the primary reactions seem to be different on going from the nucleotide level to dsDNA (Chap. 12.6). Experiments with ssODNs are quite limited.

In ssODNs containing 5BrUra, the stand break yield observed right after photolysis in aerated solution is 1/6 of that after piperidine treatment (Doddridge et

al. 1998). This is in agreement with an ALS such as 2-dRL being the major product. The nature of the direct SSB that occurs at the same site remains an open question.

A higher level of strand breakage is observed in the absence of O_2, and a markedly different pattern of 3′-termini damage is observed. It has been suggested that in the presence of O_2 Ura-5-peroxyl radicals may play a role (for its complex chemistry see Chap. 10.7), but details are not yet understood.

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12

DNA and Double-Stranded Oligonucleotides

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12.1 General Remarks

There are various agents that may induce DNA damage via free radicals (Chap. 2). The effects of ionizing radiation have been most widely studied. Here, DNA damage is induced by •OH (H• and e_{aq}^-). These reactions dominate when DNA is irradiated in dilute aqueous solution. However in cells or with DNA in aqueous solution that contain high •OH-scavenger concentration, a second phenomenon plays a role, the direct effect (ionization of DNA; see below). Ionizing radiation is deposited in small packages (spurs, tracks; Chap. 2.2) containing several radicals. This causes the formation of clustered lesions. Yet, some so-called radiomimetic drugs are also capable of producing at least two radicals in close proximity. Moreover, there are damage amplification reactions that result in more than one damaged site resulting from the attack by just one •OH radical. Fenton-type reactions (H_2O_2 + low-valent transition ions) may produce •OH site specifically, when the transition-metal ion is bound to DNA, and with this free-radical source one has to take into account that H_2O_2 and the transition-metal ions can modify the •OH-induced damage (Sect. 12.8).

The main types of damage that can be formed in DNA (base damage, apyrimidnic/apurinic (AP) site, single-strand break (SSB), double-strand break (DSB), tandem lesions and various clustered lesions) are shown schematically in Fig. 12.1. There are, however, further lesions such as DNA/DNA and DNA/protein cross-links.

As to the severity of the above lesions, it is important to note that single base damages, SSBs and even DSBs do not necessarily constitute a lethal damage (Table 12.1).

Also, in the treatment with BLM, 150 SSBs and 30 DSBs are formed at the LD_{37} level of survival (Ward 1988). It has hence be concluded that the cellular repair enzymes can cope very efficiently with these simple lesions, and much more complex lesions (clustered lesions, above the level of a DSB, see below) are required to cause a lethal event. This is supported by the observation that densely ionizing radiations show a higher RBE then low-LET radiation (Goodhead 1994).

Various tools have been used to study quite specifically DNA damage, e.g., substituting Thy by a halouracil such as 5BrUra or 5IUra. Some of the radiomimetic drugs also react with DNA very specifically and so do some inorganic radicals. Charge transfer through DNA is another aspect that leads to the preferential formation of damage at certain sites.

In cells, DNA is always surrounded by proteins (cf. Fig. 12.2). The attachment of proteins modifies the DNA damage by various effects: hampering an easy access of free-radicals, protection by compaction and repair of some of the damage by electron/hydrogen-donation. On the other hand, it may even induce some DNA damage via reactive intermediates formed upon the reaction of free radicals with the proteins. For this reason, the structure of DNA in solution and in the cellular environment will also have to be addressed.

Some of the aspects that are dealt with in this chapter have been reviewed in much more detail than it will be possible here, and reference is thus made to these reviews: the state of the art in the 1970s and 1980s (Blok and Loman 1973;

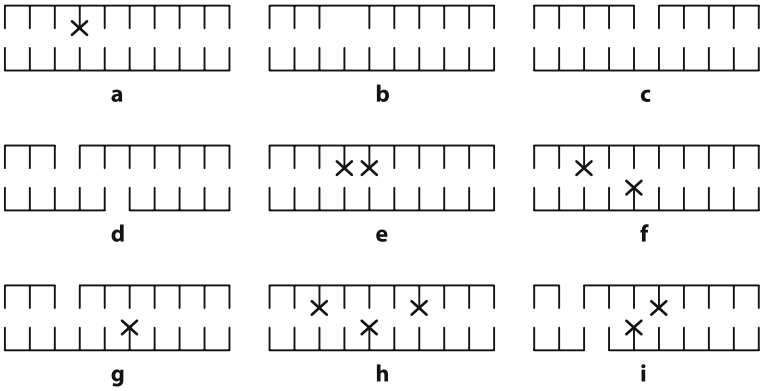


Fig. 12.1a–i Schematic representation of some types of DNA damage in dsDNA caused by ionizing radiation: **a** base damage; **b** AP site; **c** SSB; **d** DSB from two close-by SSBs; **e** tandem lesion; **f** clustered lesion with two damaged bases at opposite strands; **g** SSB with damaged base on opposite strand; **h** clustered lesion with three damaged bases; **i** clustered lesion with a DSB (from two close-by SSBs) and two damaged bases. Upon enzymatic treatment, a, b and e may turn into a SSB, while f and g may give rise to a DSB. The complex lesion h may not be recognized by the enzyme at all, nor would an enzyme treatment of i be detectable, since it already contains a DSB

Table 12.1. Some of the damage in a mammalian cell nucleus from 1 Gy of low-LET radiation. (Ward 1988; Goodhead 1994)

Initial physical damage	
Ionizations in the cell nucleus	~100,000
Ionizations directly in DNA	~2000
Excitations directly in DNA	~2000
Selected biochemical damage	
SSBs	1000
8-oxo-A (a typical single-base damage)	700
DSBs	40
DNA-protein cross-links	150
Selected cellular effects	
Lethal events	~0.2–0.8
Chromosome aberrations	~1
Hprt mutations	$\sim 10^{-5}$

von Sonntag et al. 1981; Bertinchamps et al. 1978; von Sonntag 1987), oxyl radicals plus DNA (Breen and Murphy 1995), oxidative nucleobase modifications (Burrows and Muller 1998), oxidative strand scission (Pogozelski and Tullius 1998), low-temperature EPR studies (Wyard and Elliott 1973; Hüttermann 1982, 1991; Becker and Sevilla 1998), radiation sensitizers (Brady 1980; Coleman et al. 1988), chemical nucleases (Sigman et al. 1993a), metalloporphyrins as DNA cleavers (Meunier 1992), photocleavage of nucleic acids (Armitage 1998) and anticancer agents that form DNA radicals (Lown 1985).

12.1.1 Inactivation of Viruses and Cells by Ionizing Radiation: Some General Aspects

Ionizing radiation is known to inactivate viruses and cells. In the case of viruses there are two targets, their protein coat (capsid and tail) and their nucleic acid, DNA or RNA, respectively. Viruses are inactivated, when they are no longer capable of infecting their host cells for further propagation. When the protein coat is damaged, the virus may no longer be able to attach to the cell to be infected, an effect that ranges between 5% (T1 phage; Coquerelle and Hagen 1972) and 30% (poliovirus; Ward 1980). If an attachment is still possible, the nucleic acid injection system may be impaired by protein-protein cross-linking. Injection of the nucleic acids is equally impossible when they are cross-linked to proteins such as the capsid. Any damage to the nucleic acids that is not repaired by the repair system of the host cell must lead to their inactivation. However, some viruses even carry the information for repair enzymes in their genome, but this has only been shown so far for UV-induced damage (Yasuda and Sekiguchi 1970; Furuta et al. 1997; Shaffer et al. 1999; Srinivasan et al. 2001).

When a virus suspension is irradiated in aqueous solution, the water radicals formed in the bulk solution, notably $^{\bullet}OH$ (Gampel-Jobbagy et al. 1972; Powers and Gampel-Jobbagy 1972), may react with the viruses by damaging the capsid, but they may also pass through the protein barrier and reach the nucleic acid. The structure of the virus (T7 phage: Hawkins 1978); λ phage: Georgopoulos et al. 1983; Feiss and Becker 1983) may determine which of the two processes dominates. In fact, structural effects may be quite substantial. For example, in the T-odd phages, Cu^{2+} enhances the radiation sensitivity, while in the T-even series it protects (Samuni et al. 1984). Moreover, there is an interaction of the ionizing radiation with the virus particle. The importance of this process with respect to inactivation increases with an increase in the scavenger concentration that eliminates the water radicals formed in the bulk solution (for a study comparing the sensitivity of phages to UV and ionizing radiation, see Sommer et al. 2001).

With cells, the situation is quite different. There, the DNA is not near the surface, and very reactive radicals generated in the bulk solution, such as $^{\bullet}OH$, cannot reach it. Therefore, these exogenous $^{\bullet}OH$ do not contribute to cell killing. Saturation with N_2O that doubles the bulk $^{\bullet}OH$ yield has no effect on survival (Antoku, 1983), and only the *endogenous* $^{\bullet}OH$ formed near the DNA have to be considered (Jacobs et al. 1985). These experiments already show that damage of the cell's outer membrane cannot be of major importance. Although damage to

Table 12.2. Dose (Gy) absorbed in the nucleus, cytoplasm and membrane of CHO cells at LD_{50} levels of mortality (Warters et al. 1977). ^{3}H-dThd was incorporated into the DNA, whereas ^{125}I-concanavalin was associated with the membrane. X-rays were from an external source

	Subcellular dose/Gy		
Radiation source	**Nucleus**	**Cytoplasm**	**Membrane**
X-ray	3.3	3.3	3.3
^{3}H-dThd	3.8	0.27	0.01
^{125}I-concanavalin	4.1	24.7	516.7

the outer cell membrane is of comparatively little consequence at least in mammalian cells (see below), there is a marked effect membrane composition (fluidity?) on the radiation sensitivity (Edwards et al. 1984; George et al. 1980; Yatvin et al. 1972, 1979, 1984, 1987; Yatvin 1976; ; Yatvin and Grummer 1987). Details of this most interesting effect are as yet not fully elucidated, but attention has been drawn to the fact that DNA is attached to membranes, in eukaryotic cells, for example, to the nuclear membrane. Whether the membrane-mediated damage is due to free-radical reactions such as membrane/DNA interactions, has a marked effect on repair or is due to any other process seems to be still an open question.

The view that DNA is the most important target as concerns cell killing by ionizing radiation has been derived from a microdosimetric approach (Cole et al. 1980). With eukaryotic cells that contain their DNA in the nucleus, little lethal damage is observed as long as the ionizing radiation is absorbed in the membrane or in the cytoplasm. There is a dramatic increase as soon as the ionizing radiation reaches the nucleus (Cole 1965; Munro 1970; Datta et al. 1976). DNA as the most prominent lethal target is supported by experiments, where total-cell irradiation (X-rays) is compared with the effects of radioactive nuclei emitting only short-range ionizing radiation that were placed at either the membrane or incorporated into DNA (Table 12.2).

It is seen from this table that, at the LD_{50} level, the nucleus has received about the same dose, irrespective of whether X-rays, ^{3}H-dThd or ^{125}I-concanavalin are used as the source of ionizing radiation, while the membrane has received an immense dose with ^{125}I-concanavalin and very little with ^{3}H-dThd. As expected, the cytoplasm lies in between these two extremes. Yet, irradiation of the cytoplasm (single-ion-beam experiments) is not without an effect. It may cause mutations (Wu et al. 1999) and the formation of products that induce apoptosis in nearby (unirradiated) cells (bystander effect ;Shao et al. 2004).

In the interaction of ionizing radiation with DNA one distinguishes between the *direct effect* (absorption of the ionizing radiation by DNA) and the *indirect*

effect (absorption of the ionizing radiation energy by the water that surrounds the DNA; water makes up about 70% of the cellular mass).

The *indirect effect* produces $^{\bullet}OH$, $H^{\bullet}$, and e_{aq}^{-} (Chap. 2.2), and this effect may be, and has been, studied separately from the direct effect by γ-irradiating DNA in aqueous solution. Due to its high reactivity towards any organic matter, $^{\bullet}OH$ does not travel more then about 2 Å before it is scavenged by cellular components, i.e., only those $^{\bullet}OH$ that are created in the very close neighborhood of DNA contribute to DNA damage.

The *direct effect* gives rise to a DNA radical cation ($DNA^{\bullet+}$) and an electron in addition to electronically excited DNA (DNA*) [reaction (1)]. The $DNA^{\bullet+}$ may undergo rapid hole transfer to G (GG; GGG) sites (see Sect. 12.10) in competition with other reactions.

$$\text{DNA} + \text{ionizing radiation} \rightarrow \text{DNA}^{\bullet+} + e^{-} + \text{DNA}^{*} \quad (1)$$

Moreover, there may be additional effects when the ionizing radiation is absorbed in a resonant transition such as the formation of strand breaks by K-shell excitation of phosphorous (Le Sech et al. 1996).

For mimicking the *direct effect* without a contribution of the *indirect effect*, DNA may be irradiated in frozen aqueous solution (Cullis et al. 1990). Obviously, irradiation of solid DNA is another approach that has been taken, but here the distinction between direct and indirect effect is not straightforward, since the very first water layer may be taken as an integral part of DNA. In cells, experimental data that attempt to determine the contribution of the indirect effect are based on scavenging experiments with compounds that are tolerated by cells at high concentrations (about 20%) such as DMSO or alcohols (Table 12.3). Usually concentrations ≤2 mol dm^{-3} were used in these scavenger experiments, but with glycerol the protective effect is even further increased by a factor of two upon increasing its concentration from 2 mol dm^{-3} to 5 mol dm^{-3} (Dewey 1963).

From the above data, one may take as an estimate that $^{\bullet}OH$ contribute to the lethal damage about 20% in the absence of O_2 and about 55% in its presence at low LET (for the high-LET data see Roots et al. 1985). However, there are some difficulties concerning the validity of such data. At high scavenger concentrations, the DNA structure may be considerably altered (Raaphorst and Azzam 1981), and this is likely to have an effect on the radiation response (cf. Sect. 12.11). In spores (which often are a kind of special case) DMSO *strongly sensitizes* (DMF 3–4; Ewing 1982a), and *t*BuOH in a concentration as low as 1.1×10^{-2} mol dm^{-3} is reported to reduce the radiation sensitivity of spores and cells by 50% in the presence of 1.3% O_2 but had no effect in its absence (Ewing 1981, 1982b). Moreover, photoionization of poly(U), a system that mimics the direct effect, EtOH protects against stand breakage (Schulte-Frohlinde 1986). In mammalian cells, there is a strong reduction in the release of $^{3}H_2O$ from the methyl-tritiated T moiety upon the addition of MeOH, glycerol and ethylene glycol, and yet these three scavengers show a markedly different concentration response (Roti Roti and Cerutti 1974). In addition, with a variety of *E. coli* strains *t*BuOH has only an effect on the shoulder but no effect on the slope of the inactivation curve point-

Table 12.3. Alleged contributions of •OH to the lethal action and DNA damage estimated by high concentrations of •OH-scavengers

Measured property	„•OH-contribution" in %		[Scavenger]/ mol dm^{-3}	Reference
	Hypoxia	Air		
Survival	16	67	DMSO 2.0	Millar et al. (1981)
Survival	9	44	DMSO 2.0	Skov (1984)
Survival	30	62	DMSO 3.5	Reuvers et al. (1973)
Survival		60	Glycerol 1.0	Bonura and Smith (1976)
Survival	21	54	Glycerol 2.0	Millar et al. (1981)
Survival	20	55	Ethylene glycol 2.0	Roots et al. (1982)
SSB		70	Various alcohols > 1.5	Roots and Okada (1972)
SSB	25	65	Glycerol 2.0	Millar et al. (1981)
SSB	49	66	DMSO 2.0	Millar et al. (1981)
M. luteus-sensitive sites + SSB	17	58	DMSO 3.5	Skov (1984)
DSB		59	Glycerol 1.0	Bonura and Smith (1976)

ing to an interference with the enzymatic repair rather than •OH-scavenging (Hülsewede and Schulte-Frohlinde 1986).

From this, one may conclude that it is not really possible to come up with exact numbers from experimental data, although there must be contributions from the direct and the indirect effect. Modeling using track structure calculations and reasonable assumptions concerning the DNA structure may be preferable (see, for example, Friedland et al. 1999, 2005; Bernhardt and Paretzke 2003; Bernhardt et al. 2004; structures on which such simulations are based are shown in Fig. 12.2). Of course, assumptions as to how many water molecules make up an integral part of the DNA have to be made, and this induces further uncertainties.

It seems appropriate here to mention some interesting aspects of damaging cells in aqueous solution that is not directly related to the interaction of ionizing radiation with the cellular DNA. For example, thiourea, generally considered as a protecting agent, sensitizes cells at low concentrations and this effect

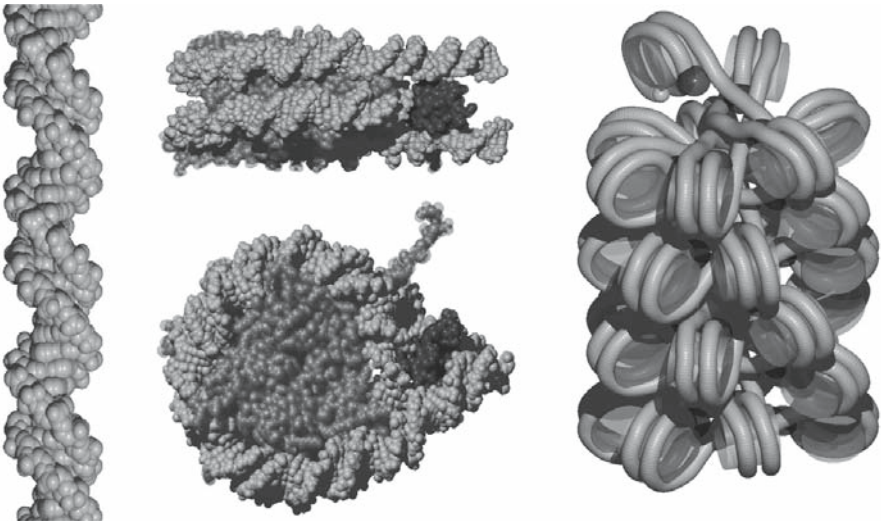


Fig. 12.2 DNA target models used for simulations. *Gray*: DNA helix; *dark gray*: histone octamer; *black* globular core of the linker histone H5. The chromatin fiber target model is represented in a simplified manner to improve the visualization of the structure. (According to Bernhardt et al. 2003, with permission)

is enhanced, when the aqueous solution is saturated with N_2O (Antoku 1983). Clearly, the reaction of $^{\bullet}OH$ with thiourea must lead to a species that is capable of inactivating cells. The intermediates that are formed have been investigated in some detail using the pulse radiolysis technique (Schuchmann et al. 2000; Wang et al. 1999). There is a host of intermediates which are all in equilibrium. They have oxidizing properties, but dGuo can only be oxidized on the pulse radiolysis time scale when deprotonated. The reduction potential of GG and GGG in DNA is markedly lower than that of a single G (Sect. 12.10), and one may speculate that such sites could be the target. At high concentrations, thiourea acts as a $^{\bullet}OH$-scavenger that prevents more severe damage, and this property then dominates.

When carrying out radiation-biological experiments in aqueous solution one has to keep in mind that γ-irradiated phosphate buffered saline media develop cytotoxic properties (Brustad and Wold 1976; Czapski et al. 1992; Saran et al. 1993). The toxic compound that is generated under such conditions is most likely HOCl, and there is the radiobiological paradox that H_2O_2 may cause DNA damage (Sect. 12.8) protects under such conditions (Saran et al. 1997). The reason for this is the ready elimination of HOCl [$HOCl + H_2O_2 \rightarrow O_2(^1\Delta_g) + HCl + H_2O$ (Held et al. 1978)] which is 1000 times more toxic than H_2O_2.

When high-energy electrons pass through a dense medium such as water, they lose a fraction of their energy as Čerenkov radiation (Redpath et al. 1981; Myasnik et al. 1980). With DNA or cells γ-irradiated in aqueous solution the UV part of the Čerenkov radiation causes the typical UV-induced DNA damage,

Table 12.4. Properties of A and G tract DNA. (Dornberger et al. 1999)

A tract	G tract
Narrow minor groove	Wide and deep minor groove
Hydration spine in the minor groove	Poor hydration in the minor groove
No amino group in the minor groove	Amino group in the minor groove
Very slow base pair dynamics	Very rapid base pair dynamics

repairable by the photoreactivating enzyme, on top of the free-radical-induced DNA damage (Vinicombe et al. 1978; Redpath and Zabilansky 1979; Moss and Smith 1980; Michael et al. 1981a; Redpath et al. 1981; Morgan et al. 1984a, 1984b; Duba et al. 1985; Morozov and Myasnik 1980; Myasnik and Morozov 1977; Myasnik et al. 1980; Skvortzov et al. 1981; for a detailed discussion, see von Sonntag 1987).

12.2 DNA Structure

Free-radical attack on DNA requires an easy access of a given radical to the site of attack. Double-stranded DNA (dsDNA) in its typical B-form is characterized by a double helix with a minor and a major groove (cf. Fig. 12.5). The B-DNA (Watson-Crick) is a right-handed double helix. Left-handed Z-DNA is generally formed with alternating GC sequences (cf. Fig. 12.6). The sugar moiety is exposed to the surface in the minor groove. The base sequence affects the size and some of the properties of the major and minor groove, especially when GC/AT pairs accumulate in a given section. Some of them are given in Table 12.4. As will be seen below, this has a considerable effect on the free-radical attack on DNA.

The water molecules at the surface of DNA are critical to the structure (Table 12.4) and play an important role in the recognition of proteins and drugs. Some of the water is released upon recognition (for a review on the dynamics of water surrounding DNA see Pal and Zewail 2004). Most tightly bound are two water molecules per nucleotide subunit that solvate the phosphate groups. Less firmly bound are the four water molecules that form hydrogen bridges with the oxygens of the sugar moiety. About five interact with the bases in the major groove. When the DNA is frozen, all these water molecules do not assume an ice-like structure, and only the secondary water shell (>14 water molecules per nucleotide) freezes like normal ice (Ebert 1980; for the water content of DNA as a function of ambient humidity see Lett and Alexander 1961).

As the present book is concerned with the free-radical chemistry of DNA, it is worth mentioning that hydroxyl radical footprinting, based on the forma-

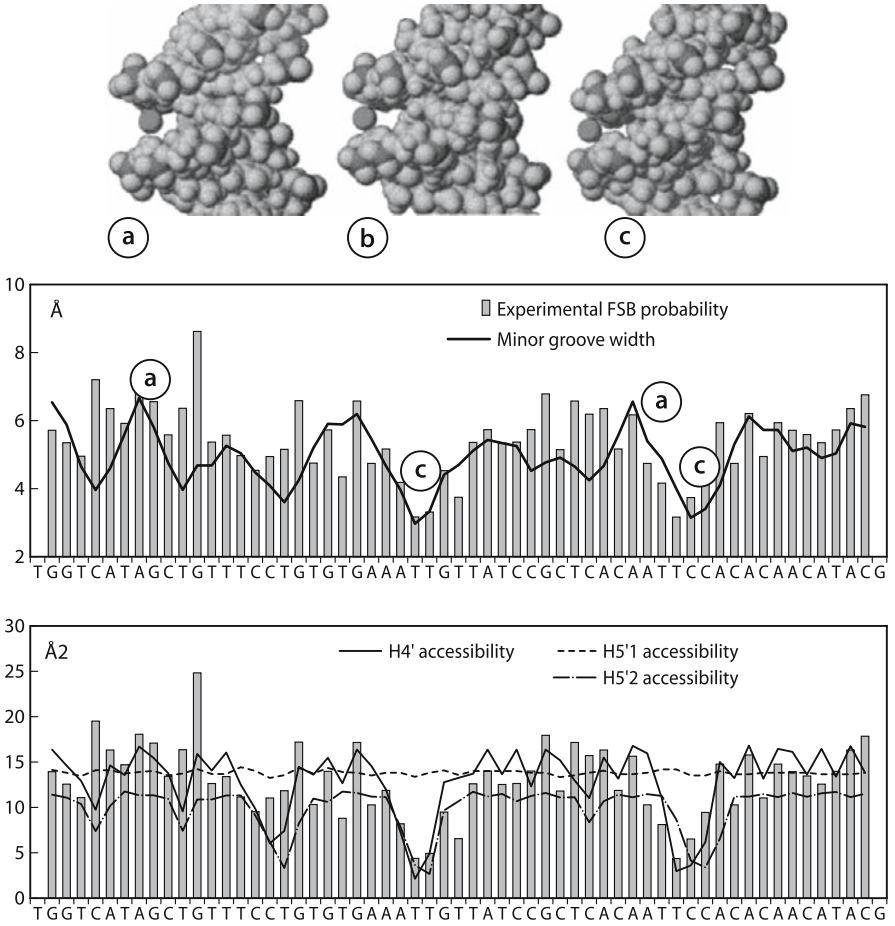


Fig. 12.3. Frequency of frank SSB induction, size of the minor groove and accessibility of H4′ and H5′ of the sugar moieties in a model of a 68 bp B-DNA fragment according to Spotheim-Maurizot et al. (2003, with permission). *Top* Penetration of •OH into the minor groove when the size of the groove is large (*a*), middle (*b*) and small (*c*). *Middle* formation of frank SSBs upon •OH-attack as a function of the position along the DNA fragment (experimental data are shown as a histogram) superimposed on the calculated variation of the minor groove width along the DNA sequence (full line). *Bottom*: accessibility of H4′, H5′1 and H5′2 along the DNA sequence (*full lines*) superimposed on the frequency of frank SSB formation (*histogram*)

tion of strand breaks by this very reactive radical (see below), has been used with advantage to probe DNA-protein contacts (Tullius et al. 1987; Bashkin and Tullius 1993; Tullius 1991, 1996; Levin et al. 1991; Hayes et al. 1990a; Price and Tullius 1992; Franchet-Beuzit et al. 1993; Draganescu and Tullius 1996). For example, this approach allowed to analyze the structures of Holliday junctions (Churchill et al. 1988; Kimball et al. 1990) which are ephemeral intermediates in genetic recombination. This technique also yielded valuable information as

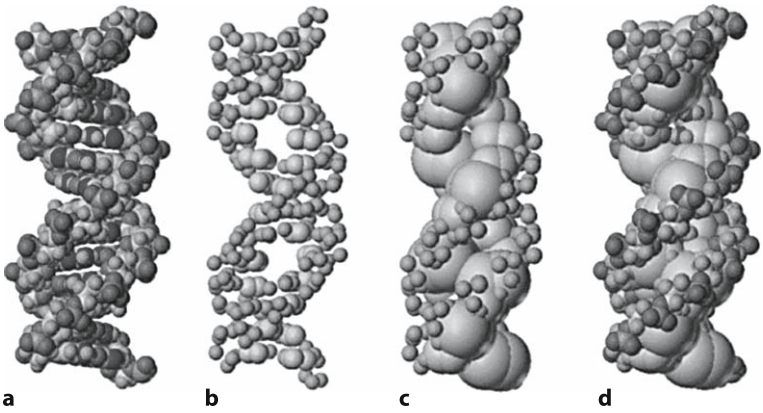


Fig. 12.4a–d. RADACK procedure: **a** B-DNA represented in a space-filling model; **b** the reactive atoms only; **c** the same atoms but with sizes according to their •OH cross-section; **d** with the non-reactive atoms re-added according to Begusova et al. (2001b, with permission)

to the structure of DNA in nucleosomes (Hayes et al. 1990b, 1991; Bashkin et al. 1993), bent DNA (Tullius and Burkhoff 1988) and mapping the gene regulation domains (Spotheim-Maurizot et al. 1995b). A similarly unspecific cleaving agent for probing DNA-protein contacts seems to be the photoexcited uranyl ion (Nielsen et al. 1988). Intercalating drugs may be much more selective in their binding and hence in their cleavage reaction. As an example may serve photoexcited acridine derivatives (Jeppesen et al. 1988).

12.2.1 Accessibility of the Sugar Moiety to Radical Attack and Probability of Damage Induction

From the above, it is obvious that, beyond a global modulation of accessibility by bound molecules such as protection by proteins, structural details of the DNA determine the accessibility of radicals to sugar moieties. Among others, accessibility will determine the H-abstraction from the various C-H sites of the sugar moiety even by the highly reactive •OH. Its overall relative accessibility, defined as the accessible surface determined by rolling a sphere simulation of an •OH onto the van de Waals surface of the H atoms, calculated for a molecular model of a 68 bp DNA in B-form, shows that H5′1 is the most exposed one, followed by H5′2 and H4′; intermediate access is observed for H2′2 and H3′, and the most hidden ones are H2′1 and H1′ (Sy et al. 1997; Begusova et al. 2001b).

A 80-bp DNA fragment has been used to study in detail the relation between DNA damage (frank SSBs induced by •OH attack on the sugar moiety, notably at H4′ and H5′) and the accessibility of the H atoms involved in breakage (Sy et al. 1997). The experimental results were compared to the calculated accessibility of the H atoms, and a good fit was observed between the frequency of SSBs at each

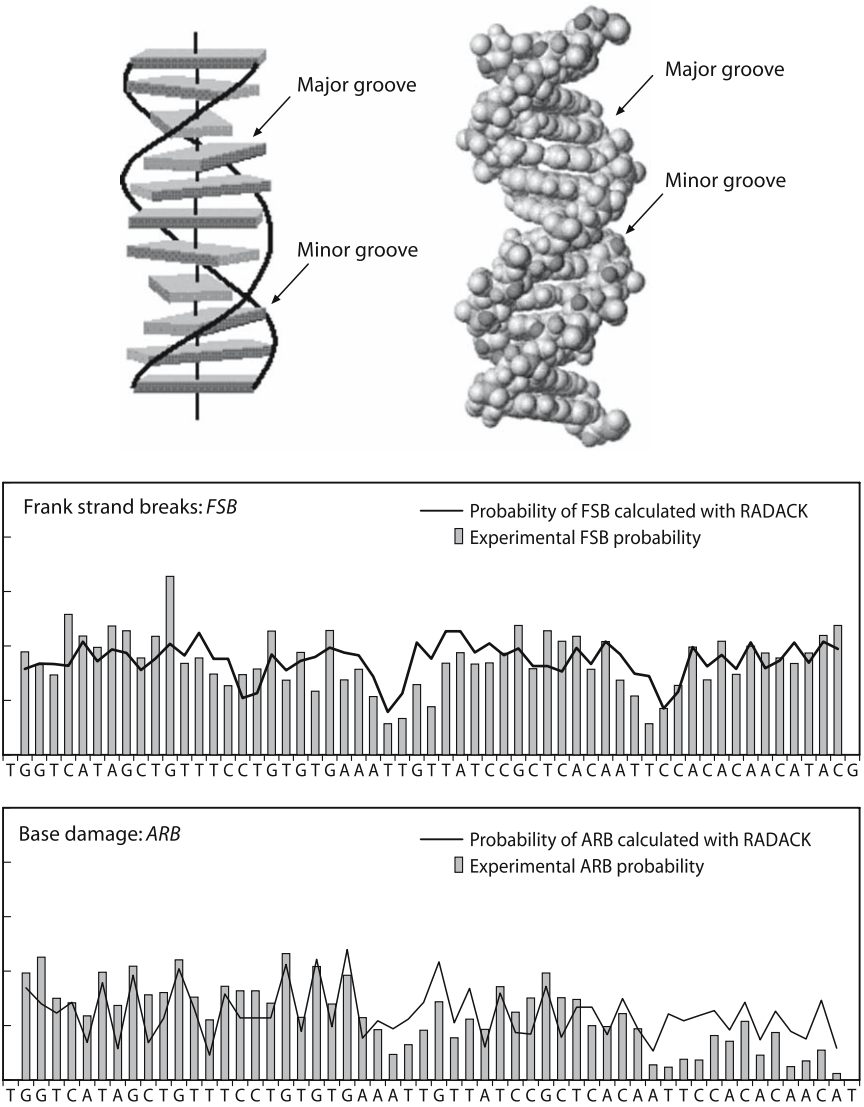


Fig. 12.5. Radiolysis of B-DNA. Frequency of frank SSBs (*top*) and damaged bases (ALSs, *bottom*). *Solid lines* were calculated using RADACK procedure according to Spotheim-Maurizot et al. (2003, with permission)

nucleotide site and the accessibility to H4′ and H5′2 atoms of each nucleotide. Both patterns exhibit also this strong sequence dependence. Moreover, the width of the minor groove measured on the molecular model of the DNA of the same sequence presents the same sequence dependence (Fig. 12.3). This is due to the

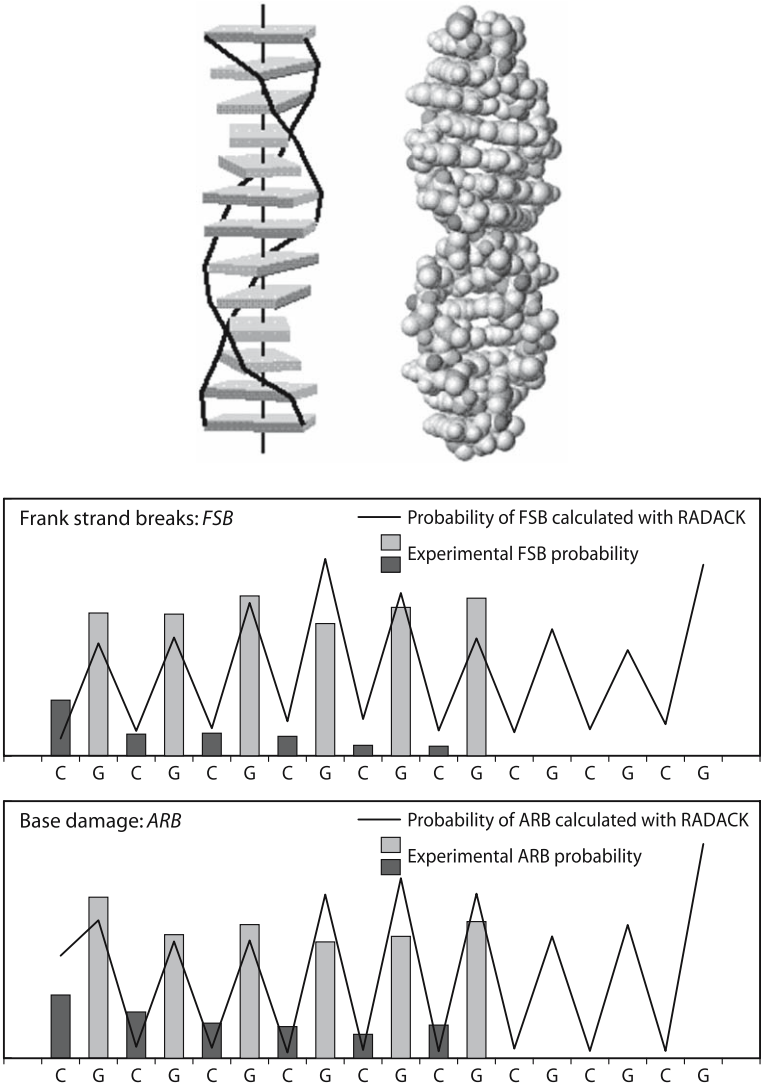

Frank strand breaks: *FSB*
Probability of FSB calculated with RADACK
Experimental FSB probability
C G C G C G C G C G C G C G C G C G
Base damage: *ARB*
Probability of ARB calculated with RADACK
Experimental ARB probability
C G C G C G C G C G C G C G C G C G

Fig. 12.6. Radiolysis of Z-DNA. Frequency of frank SSBs (*top*) and damaged bases (ALSs, *bottom*). *Solid lines* were calculated using RADACK procedure according to Spotheim-Maurizot et al. (2003, with permission)

fact that the size of the minor grove in B-DNA varies substantially with the base sequence and influences markedly the accessibility to H4′ and H5′2 but barely that to H5′1. When the width of the minor groove is <4.5 Å, the accessibility of H4′ and H5′2 drastically diminishes and so does the frequency of induced frank SSBs.

Simulation of frank SSBs and base damage as expressed by ALS has been achieved with the RADACK (RADiation attACK) procedure (Begusova et al. 2001b). This takes into account that the various nucleobases and the hydrogens of the sugar moiety react with different rate constants. The effect is shown in Fig. 12.4, where B-DNA is represented in a space-filling model with only the reactive atoms represented, with the same atoms but with sizes according to their •OH cross-section or with the non-reactive atoms re-added. It is the last structure that •OH "encounters" in the RADACK procedure.

The variations in the probability of damage induction calculated with RADACK are well reflected in the variation of frank SSBs and damaged bases (as expressed by ALS) for different forms of DNA: B-DNA (Fig. 12.5), Z-DNA (Tartier et al. 1994; Fig. 12.6) and for a DNA quadruplex (Tartier et al. 1998). The yield of •OH-induced frank SSBs of various topoisomers of DNA minicircles is the same (Culard et al. 1994). It has been concluded that the accessibility of H4′ is already sufficient in the relaxed topoisomer that an increase in accessibility in the T-2 topoisomer would be without effect.

For further modeling studies of this group see Begusova et al. (1999, 2000a, b, 2005); Michalik et al. (1995a–c); Savoye et al. (1996); Sy et al. (2001); Tartier et al. (1994).

12.3 Single Base Damage

12.3.1 General Remarks

Nearly all modifications that have been detected on the model level (Chap. 10) are also found in free-radical damaged DNA. Obviously the DNA-bound lesions are much more difficult to detect, and there is an ongoing discussion as to the best procedure of their excision (Chap. 13.2; for a review on the excision and repair of base lesions in vivo see Wallace, 2002). Mechanistic details concerning the formation of the base lesions have been discussed in Chapters 10 and 11, and only some additional information will be given below and in the section on clustered lesions where the phenomenon of tandem lesions, two damaged bases that are formed side by side, is dealt with. The yields of damaged bases formed upon γ-irradiation in aqueous solution, as has been determined by the GC-MS/SIM technique, are compiled in Table 12.5.

As can be seen from this table, the detectable products amount to 35% of •OH at most. Moreover, dsDNA gave rise to markedly lower yields than ssDNA. Whether this is due to incomplete •OH scavenging in these systems due to low-molecular-weight impurities is as yet unknown. Obviously, as we know from other studies, there are more products formed such as Iz, Z, cA, cG, 5HmU, 5ForU, Fo and hydantoin lesions (see below) than have been determined in this study. Moreover, there is an attack of •OH at the sugar moiety that is generally believed not to exceed 20% by much (see, however, Sect. 12.4.4). Thus, there is a gap in the material balance. The material balance is especially poor in the absence of O_2.

Table 12.5. Compilation of products and their *G* values (unit: 10^{-9} mol J^{-1}) in the radiolysis of ss- and dsDNA in aqueous solution as determined by the GC-MS/SIM technique (Fuciarelli et al. 1990). For further data under air, see reference

Product	N_2		N_2O		N_2O/O_2	
	dsDNA	ssDNA	dsDNA	ssDNA	dsDNA	ssDNA
5,6-H_2Thy	7.8	30.3	5.6	8.8	n.d.	n.d.
5OH5,6H_2Thy	3.8	26.0	7.7	40.5	<0.1	<0.1
Tg	3.8	6.6	10.2	14.3	43.4	58.4
5OH5,6H_2Cyt	2.0	5.4	2.6	7.4	<0.1	<0.1
Cg	2.6	13.3	10.7	16.3	25.6	32.2
FAPY-A	2.9	1.3	8.7	3.1	5.9	4.5
8-oxo-A	0.9	0.5	5.3	3.1	15.8	24.0
FAPY-G	6.8	1.6	12.4	4.1	3.6	4.9
8-oxo-G	<0.1	<0.1	16.2	7.5	46.7	70.5
Sum	31	87	79	105	141	195
Yield with respect to $^{\bullet}$OH	11%	31%	14%	19%	25%	35%

n.d., Not detectable

Under such conditions neither the Fo, 5HmU, 5ForU nor the hydantoin lesions are formed. Thus, the fact that such compounds cannot be determined with the given technique cannot serve as an 'excuse' for the poor material balance of only 19% with respect to $^{\bullet}$OH. In model systems, dimers are major products (Chap. 10.3). Applied to DNA, the formation of dimers would constitute either an intra-strand or an inter-strand cross-link. This possibility has usually been disregarded, since DNA being rod-like is not very flexible. Radical-radical recombination could occur, however, at a distance, and from other polymers there is evidence that intra-molecular reactions do occur also at a distance (Chap. 9.4). The type of reaction that is envisaged here would form a small loop and would not be connected with an increase in molecular weight. A DNA-DNA cross-link that connects two DNA molecules, and thus increases the molecular weight, is a much less likely reaction if other polymers are a good guide (Chap. 9.6).

It is noteworthy that some product yields do not change very much whether the solutions were saturated with air or with N_2O/O_2, despite the fact that the $^{\bullet}$OH yield is halved in air-saturated solutions (cases in point are for ssDNA and in the units of Table 12.5: Cg (37.9), Tg (43.4), 8-oxo-G (62.0); for further values see Fuciarelli et al. 1990). Whether this means that $O_2^{\bullet -}$ that is an abundant and freely diffusing radical under such conditions plays an important role in the

Table 12.6. γ-Radiolysis of suspended mamalian chromation in aqueous solution under different gassing conditions. *G* values in units of 10^{-9} mol J^{-1}

Product	Ar	N_2O	Air	N_2O/O_2
5,6H_2T	0.48	0.26	n.d.[a]	n.d.
5OH5MeHyd	[b]	b	0.21	0.42
5OHHyd	[b]	[b]	[b]	0.22
Cg	0.5	0.95	1.1	2.3
5OH5,6H_2T	0.3	0.3	n.d.	n.d.
5OH5,6H_2Cyt	0.17	0.17	n.d.	n.d.
HmU	0.045	0.064	0.02	0.052
Tg	0.045	0.094	0.18	0.40
5OH6OHC	[b]	[b]	0.14	0.33
FAPY-A	0.82	0.96	0.60	1.02
8-oxo-A	0.45	0.80	1.10	3.50
FAPY-G	0.96	1.81	1.11	1.81
8-oxo-G	0.55	1.35	3.85	8.05

[a] n.d., Not detected
[b] No increase in the amount above the background level at doses up to 200 Gy

formation of these products has not yet been investigated. This effect has not been observed in the case of chromatin irradiations (Table 12.6), and this must be taken as a caveat not to overinterprete these data.

With chromatin suspensions, DNA product yields are an order of magnitude lower as compared to DNA solutions. This is partially due to •OH-scavenging by the nucleoproteins, but since one deals here with suspensions, •OH-scavenging by dissolved impurities has an even more dramatic effect than in DNA solutions. Moreover, some of the DNA damage may be repaired by the surrounding protein, but the existing data do not provide a firm conclusion concerning this point.

The main detectable DNA lesions, as damaged bases are concerned, were also found in irradiated cells. Table 12.7 compiles data that were obtained with γ (low LET) and carbon ion (high LET) radiations.

As is seen from the table, the yield of detectable damaged bases is noticeably lower for the high LET radiation despite its higher RBE. This agrees with the concept of clustered lesions being responsible for lethality (for the repair of clustered lesions see Dianov et al. 2001).

Table 12.7. Yields of damaged bases (unit: $(10^9 \text{ bases})^{-1}$ Gy^{-1}) formed in monocyte cells upon γ and carbon ion irradiation. (Pouget et al. 2002)

Product	γ	Carbon ion
Tg	97	62
8-oxo-A	3	3
FAPY-A	5	1
8-oxo-G	20	10
FAPY-G	39	22
5HmU	29	12
5ForU	22	11
5-OHdUrd	<0.2	<0.2

12.3.2 Products Derived from the Thymine Moiety

The products derived from T that have been detected in free-radical damaged DNA are depicted in Fig.12.7.

Tg, 5OH5,6H_2T, 5,6H_2T, 5HmU and 5OH5MeHyd are readily detected by GC/MS after excision from the damaged DNA and trimethylsilylation (Chap. 13.2). For the Tg lesion, an ultrasensitive assay is also available that even allows its detection in cells at a dose as low as 0.25 Gy and to follow its repair (Le et al. 1998).

In model systems, there is always some ForUra besides HMUra, and there is no reason, why it should not be formed in DNA as well. In model systems including ODNs, the Fo lesion, also originating from C (see below), is of major importance. It must also be formed in DNA but cannot be determined by the GC/MS technique. Under certain conditions, a treatment with repair enzymes may form a SSB that can be taken as an indication of its presence. Yet, P1 nuclease, for example, cleaves well that lesion in d(GpFo) but not in d(FopG), d(FopA), d(FopT), d(FopC) (Falcone, Box, 1997). The tandem lesion d(Fop8-oxoG) is also not hydrolyzed under these conditions.

The attack of •OH at the methyl group of T was first followed by the release of ^3H_2O from DNA ^{3}H-labeled at this position (Swinehart and Cerutti 1975). With ΦX174 DNA γ-irradiated in aqueous solution and also within the phage an in situ protection factor of 9.4 was noticed, and for *E. coli* the corresponding value was 790. Interestingly, for the formation of the Tg lesion, the protection factors were noticeably lower, 1.3 and 140, respectively. In the phage, 200 of the latter lesions are formed at a dose equivalent to 37% survival as indicated by

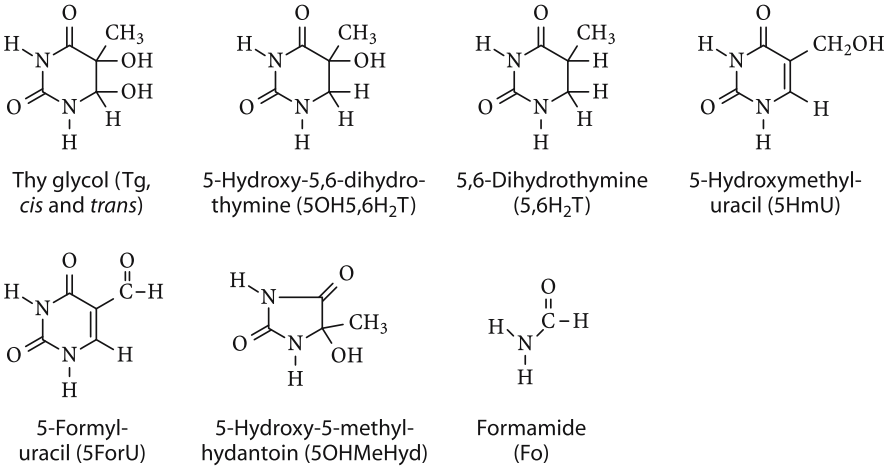


Fig. 12.7. Products derived from T detected (or most likely formed, see text) in free-radical-damaged DNA

the plaque forming ability. Thus considerable repair must occur during this assay.

As expected (Chaps 10.3 and 10.4), 5,6H$_2$Thy and 5OH5,6H$_2$Thy are only formed, when O_2 is absent (Table 12.5).

12.3.3 Products Derived from the Cytosine Moiety

The products derived from C that have been detected in free-radical damaged DNA are depicted in Fig. 12.8.

γ-Irradiated phage DNA ^{14}C-labeled at C yielded Ug as the main labeled product after acid hydrolysis (Ayaki et al. 1987). A more recent study on DNA γ-irradiated in air saturated aqueous solutions reports the formation of Ug ($G = 0.021 \times 10^{-7}$ mol J^{-1}), 5OHC ($G = 0.025 \times 10^{-7}$ mol J^{-1}) and 5OHU ($G = 0.036 \times 10^{-7}$ mol J^{-1}; in total about 3% of •OH; Wagner et al. 2004). These products are thought to have the Cg lesion as precursor. In vivo, the deaminated Cg lesions cause C→T transitions (Kreutzer and Essigmann 1998) as does the 5OHC lesion (Feig et al. 1994). The Fo lesion can also have T as a precursor.

12.3.4 Products Derived from the Adenine Moiety

The products derived from A that have been detected in free-radical damaged DNA are depicted in Fig. 12.9.

A ^{32}P-postlabeling assay has been developed for the cA lesion (Randerath et al. 2001), and interesting data as to their elimination from DNA indicate that this lesion is not repaired base excision repair (BER) as most other damaged

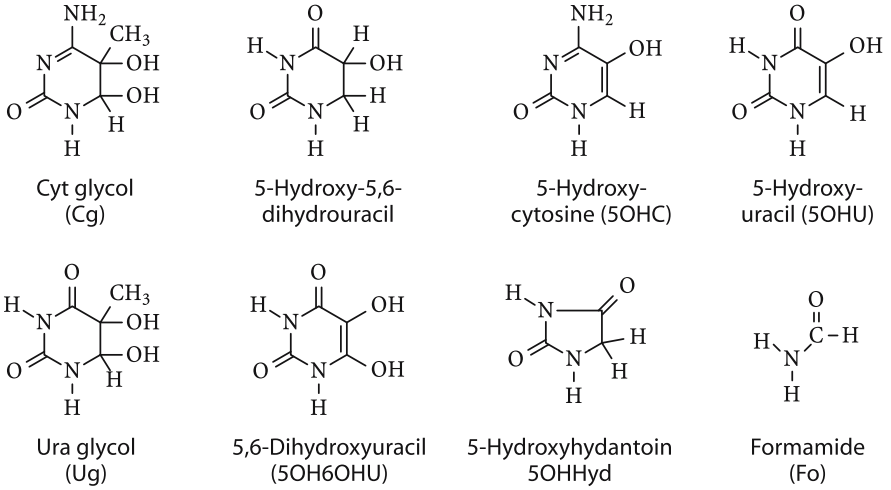


Fig. 12.8. Products derived from C detected (or most likely formed, see text) in free-radical-damaged DNA

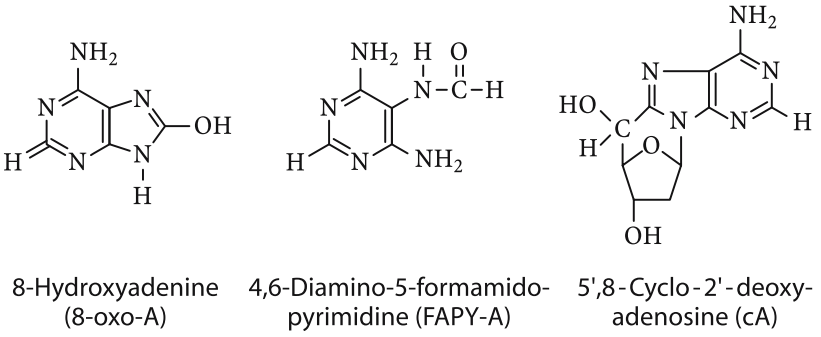
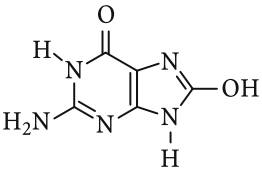


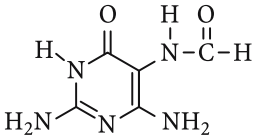
Fig. 12.9. Products derived from A detected in free-radical-damaged DNA

base sites but requires nucleotide excision repair (NER). This study has been extended to various biochemical aspects of the effects of this lesion such as gene transcription (Marietta et al. 2002) and the ability of various enzymes to excise this lesion (Jaruga et al. 2004). In the latter study, it has been shown that the cA lesion can be fully released by a cocktail consisting of nuclease P1, snake venom phosphodiesterase and alkaline phosphatase which is in some contradiction to results obtained with a similar, but not identical, enzymatic cocktail (Romieu et al. 1999a).

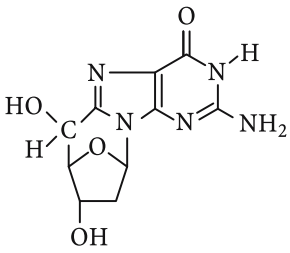
In γ-irradiated N_2O-saturated DNA solution, 0.56 cA lesions per 10^6 bases are formed per Gy (Dizdaroglu et al. 2001b). This is equivalent to a *G* value of 0.9×10^{-9} mol J^{-1}. The yield of the cA lesion is hence considerably lower than that of



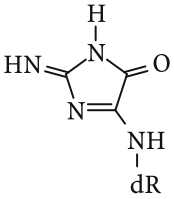
8-Hydroxyguanine
(8-oxo-G)



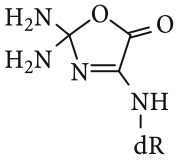
2,6-Diamino-4-hydroxy-5-
formamidopyrimidine
(FAPY-G)



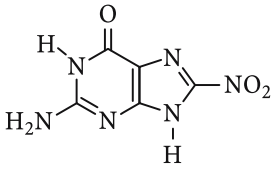
5',8-Cyclo-2'-deoxy-
guanosine (cG)



Imidazolone
(Iz)



Oxazolone
(Z)

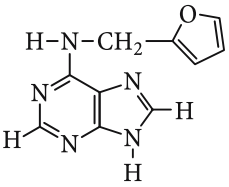


8-Nitroguanine

Fig. 12.10. Products derived from G detected in free-radical-damaged DNA

the corresponding cG lesion (see below). The *S* diastereoisomer of the cA lesion may be more cytotoxic than the *R* diastereoisomer (Kuraoka et al. 2001).

N(6)-furfuryladenine (kinetin) is formed in vivo, excreted in the urine, and has been assumed to be a secondary, free-radical-induced DNA product (Barciszewski et al. 1996, 1997, 1999, 2000; Wyszko et al. 2003).



Kinetin

Details concerning its formation will have to be elucidated.

12.3.5 Products Derived from the Guanine Moiety

The products derived from G that have been detected in free-radical damaged DNA are depicted in Fig. 12.10.

The product yields in γ-irradiated DNA are given in Tables 12.5-12.7. FAPY-G has always been observed, but 8-oxo-G yields were extremely low, when γ-irradiations were carried out under N_2. This may serve as a caveat for the common practice to use 8-oxo-G as a kind of 'marker' for free-radical DNA damage (for assays see Chap. 13.2). Besides the other products reported in Table 12.5, 8 cG lesions per 10^6 bases are formed per Gy in γ-irradiated N_2O-saturated DNA (50 μg ml^{-1}) solution (Dizdaroglu et al. 2001a; corresponding to 1.6×10^{-9} mol J^{-1}), and this lesion has also been observed in γ-irradiated cultured human cells (Dizdaroglu 1986; for its elimination by nucleotide excision repair see Kuraoka et al. 2000).

G has the lowest redox potential among the nucleobases (Chap. 10.2 and Sect. 12.10). Inorganic radicals that have a sufficiently high oxidation potential (Chap. 5) may be used to oxidize specifically G. One of them is $SeO_3^{\bullet -}$. Its rate constant with DNA has been determined at 3×10^7 dm^3 mol^{-1} s^{-1} (Martin and Anderson 1998), about one order of magnitude slower than the rate constant of $^{\bullet}OH$ ($k = 4.5 \times 10^8$ dm^3 mol^{-1} s^{-1}; Udovicic et al. 1994). Upon one-electron oxidation $G^{\bullet +}$ is formed which subsequently deprotonates yielding $G^{\bullet}$. Due to hole transfer within DNA, $G^{\bullet}$ is possibly the most abundant DNA radical (Sect. 12.10). It does not react with O_2 and thus can persist for a long time in oxygenated solutions (Chap. 10.2). It may be reduced (repair of DNA damage) by compounds having a lower reduction potential (Milligan et al. 2001b), and for the reduction by tryptophan a rate constant of 10^7 dm^3 mol^{-1} s^{-1} has been given. The rate of repair by substituted phenols varies with their reduction potentials, and, for example, 4-cyanolphenol ($E^7 = +1.17$ V) reacts with 7.3×10^5 dm^3 mol^{-1} s^{-1} while 4-aminophenol ($E^7 = +0.41$ V) reacts with 4.7×10^9 dm^3 mol^{-1} s^{-1} (Milligan et al. 2004). The rate constant of the DNA-binding drug *Hoechst 3358* with $G^{\bullet}$ is 1.7×10^9 dm^3 mol^{-1} s^{-1} (Table 12.17; for its protection of DNA, see Sect. 12.11).

As with other reducing agents, $G^{\bullet}$ reacts with $O_2^{\bullet -}$ (Chap. 10.2) which is the most abundant freely diffusing peroxyl radical. Upon two-photon excitation of a 2-aminopurine-containing ss- and dsODN in air-saturated solutions, photoionization leads to the formation of e_{aq}^- (and subsequently $O_2^{\bullet -}$) and the 2-aminopurine radical cation oxidizes a neighboring G (leading to $G^{\bullet}$ plus H^+; Misiaszek et al. 2004). $G^{\bullet}$ and $O_2^{\bullet -}$ react with one another (ssDNA: $k = 4.1 \times 10^8$ dm^3 mol^{-1} s^{-1}; dsDNA: 2.7×10^8 dm^3 mol^{-1} s^{-1}). In the majority of these events G is reformed, but with an efficiency of 15% Iz and, to a minor extent, 8-oxo-G are formed. The suggested mechanism is shown in Chapter 10.2.

In the reactions of peroxynitrite, $CO_3^{\bullet -}$ plays a major role (Chap. 2.2). In the self-complementary ODN d(AACGCGAATTCGCGTT) it reacts ($k = 1.9 \times 10^9$ dm^3 mol^{-1} s^{-1}) exclusively with G (by ET) inducing an alkali-labile lesion (Shafirovich et al. 2004). When $NO^{\bullet}$ and $O_2^{\bullet -}$ is generated simultaneously as precursors of peroxynitrite that forms $^{\bullet}OH$ and $^{\bullet}NO_2$ upon its decay and when CO_2 is present $CO_3^{\bullet -}$ and $^{\bullet}NO_2$ (Chap. 2.4), formation of 8-oxo-G is observed (Inoue and Kawanishi 1995) and also upon peroxynitrite addition to DNA (Douki and Cadet 1996). In the latter experiments, the effect of peroxynitrite was compared with that of ionizing radiation, but although the peroxynitrite concentration applied was 1000 times the radiolytic $^{\bullet}OH$ yield, the 8-oxo-G and Z yields were markedly lower. Only very low 8-oxo-G yields were also detected in another study

(Yermilov et al., 1995), and this may most likely be due to its rapid degradation by peroxynitrite (Uppu et al. 1996). 8-Nitroguanine is a typical peroxynitrite-induced product, but to become prominent CO_2 has to be present (Yermilov et al. 1996). On the other hand, CO_2 inhibits SSB formation by suppressing the $^{\bullet}OH$ route in peroxynitrite decomposition (Chap.2.2). The 8-nitroguanine lesion is sensitive to hydrolysis, and, in phosphate buffer at 37 °C, it is eliminated from DNA within a couple of hours (Yermilov et al. 1995; for a review on the biological effects peroxynitrite-induced DNA damage see Szabo and Ohshima 1997).

When methyl radicals (formed in the reaction of Fe^{2+}/EDTA/H_2O_2 in the presence of DMSO; Chaps 2.5 and 3.2) were reacted with RNA, considerable methylation at the purines was observed (formation of 8MeGua, 2MeAde and 8MeAde; Kang et al. 1993). Surprisingly, the 2-methyladenine/8-methyladenine ratio strongly falls upon increasing the pH from 5.2 to 9.2.

Peroxyl radicals, generated in the photolysis of aerated solutions of adequately functionalized acetophenones, oxidize selectively the G a high preference in the formation of 8-oxo-G as compared to Z/Iz (Adam et al. 2001b). Whereas 8-oxo-G (in experiments with dGuo) is further oxidized to 4-HO-8-oxo-G by peroxyl radicals (Chap. 10.13), the 8-oxo-G lesion within DNA seems to be much more stable against peroxyl radical attack (Adam et al. 2001a). The 8-oxo-G lesion can serve as a sink for oxidative damage in DNA (Sect. 12.10.3). The radical that is formed reacts readily with $O_2^{\bullet -}$ (ssDNA: 1.3×10^8 dm^3 mol^{-1} s^{-1}; dsDNA: 1.0×10^9 dm^3 mol^{-1} s^{-1}), leading mainly to dehydroguanodinohydantoin lesions (Misiaszek et al. 2005).

12.4 Sugar Damage

12.4.1 General Remarks

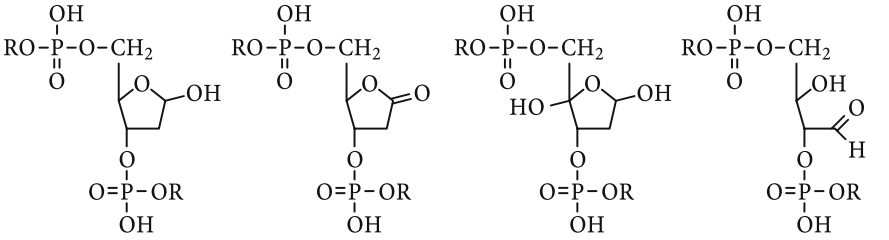
When the sugar moiety is damaged, important lesions such as abasic sites and strand breaks are induced. In the course of these reactions, unaltered bases are released. This may be considered as a loss of information, but the situation seems to be much more complex than that (Kroeger et al. 2004a). Some aspects of $^{\bullet}OH$ attack on the sugar moiety in competition to attack at the bases have already been dealt with above. Here, the products and the reactions that are leading to these products will be discussed. In poly(U) and poly(C) damage amplification reactions involving the sugar moiety do occur (Chap. 11.2; for corresponding reactions in other polymers see Chap. 9.4). On the dinucleotide level such reactions are also observed (Chap. 10.3), but for DNA strong evidence is still missing.

12.4.2 Abasic Sites

As the word suggests, an abasic site (apurinic/apyrimidinic site, AP) lacks the base, but the DNA backbone is more or less intact. The simplest AP is a 2-dR site, formed

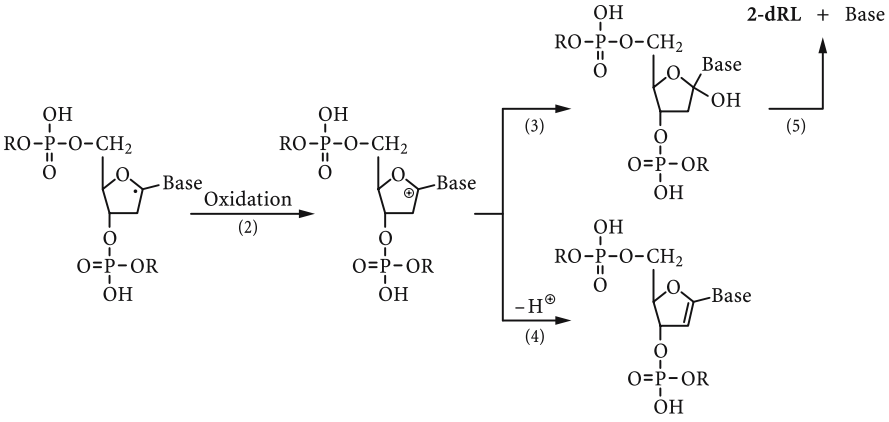
upon the loss of a nucleobase. Hydrolytic depurination creates such a site (3.3 2-dRs at pH 5.2 at 70 °C per h in pRSVneo plasmid DNA; Weinfeld et al. 1990), but it is difficult to visualize such a site to be formed by free-radical reactions. Typically, a fraction of the damaged base is still attached at the sugar moiety (for example, the Fo lesion). On the nucleoside model level, 2-deoxyribose would have to be formed. Thus far this has never been reported. Yet, subsequent hydrolytic treatment can eliminate some of the damaged bases and create an 2-dR AP (see below).

For 2-dRL, the *C*(4′)-oxidized lesions (C4′-AP) and the lesion that has lost *C*(1′) (Erythrose-AP) is strong experimental evidence.



2dR and 2-dRL are in equilibrium with their ring-open forms, and thus all these lesions can carry an OH group in β-position to a phosphate group. Like RNA (OH group at *C*(2′)], such structures are alkali labile and release the phosphate group upon treatment with alkali (Brown and Todd 1952; Chap. 13.2). For example, this procedure was used for the detection of 2-dRL in γ-irradiated DNA (Dizdaroglu et al. 1977). At the time, it was not yet known that 2-dRL also gives rise to 5-MF under such conditions (see below), and the reported yield ($G = 0.01 \times 10^{-7}$ mol J^{-1} in N_2O/O_2-saturated solutions and $G = 0.005 \times 10^{-7}$ mol J^{-1} in N_2O-saturated solutions) must be on the low side.

The precursor of the 2-dRL lesion is the *C*(1′). The carbocation formed upon its oxidation [reaction (2)] could react with water giving rise to a labile hemiaminal [reaction (3)] that would readily hydrolyze giving rise to the 2-dRL lesion and an unaltered base [reaction (5)].



In competition, the carbocation may lose a *C*(2′) proton [reaction (3)]. The resulting species is stable against hydrolysis, and such a lesion should persist in DNA (Chen and Greenberg 1998). This species could also be formed in a disproportionation reaction.

The 2-dRL lesion is prone to β-elimination (Hwang et al. 1999a), and under mild alkaline conditions or in the presence of other catalysts gives rise to a strand break (see below and Sect. 12.9). Its repair by cellular enzymes has been studied in some detail, and it has been concluded that this lesion has a considerable mutagenic potential (Kroeger et al. 2004a).

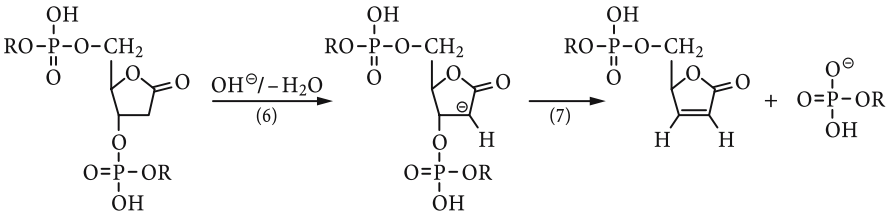
C4′-AP is formed whenever the *C*(4′) radical is oxidized in competition to strand breakage by phosphate release in β-position (Sect. 12.4.4). For example, C4′-AP accounts for ~40% of the DNA lesions produced by BLM. The action of cellular repair enzymes on this lesion has been studied in some detail, and it has been concluded that this lesion should play a significant role in the cytotoxicity of BLM (Kroeger et al. 2004b; see also Greenberg et al. 2004a).

The Erythrose-AP, resulting from a *C*(2′) oxidation, has been incorporated into specific DNA sites (Greenberg et al. 2004b). Details of the processing of this lesion by repair enzymes has been studied. One aspect, among others, is the observation that under certain conditions A is incorporated preferentially opposite to this allegedly non-coding site.

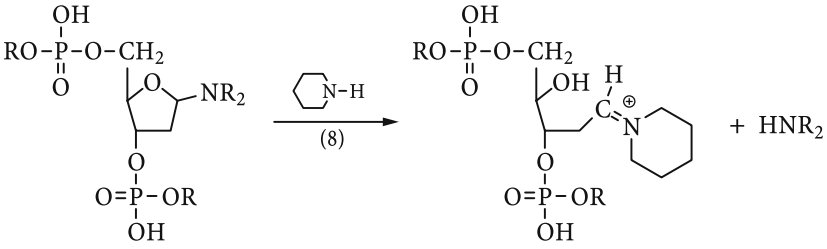
The thermodynamic stability (melting) of DNA containing an AP is markedly reduced (Gelfand et al. 1998). In a 13-mer DNA duplex, this lesion does not alter the global B-form conformation, but it induces a significant enthalpic destabilization of the duplex the amount of which being strongly dependent on the base sequence.

12.4.3 Alkali-Labile Sites

Alkali-labile sites (ALS) may contain an AP such as 2-dRL or certain damaged bases that are released from the sugar moiety upon treatment with alkali (OH^- or an organic base such as piperidine). Subsequent to this, a strand break is induced, and this procedure is often used to detect damaged bases within DNA. As the mechanism of the decomposition of 2-dRL by alkali is concerned, it is been suggested that the carbonyl function at *C*(1′) acidifies H2′ and deprotonation [reaction (6)] leads to a β-elimination of the phosphate group [reaction (7)].



Piperidine that is typically used for this purpose has a double function. As a nucleophile, it releases the damaged base (denoted as HNR_2) and assists in the acidification of the H2′ by forming a positively charged Schiff base [reaction (8)].



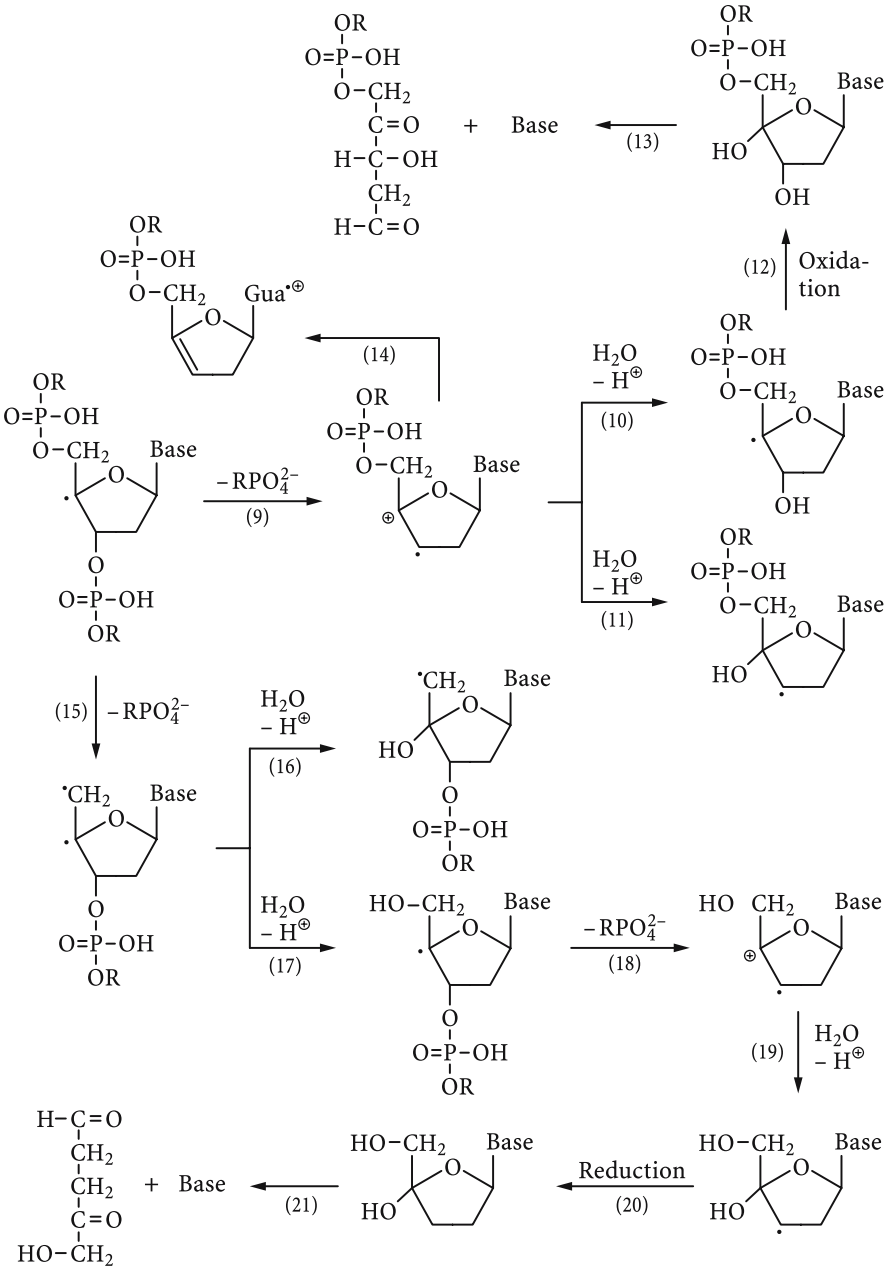
The kinetics of the cleavage of the 2-dRL lesion is by β-elimination has been studied in some detail as a function of pH and temperature for a number of ODNs of varying length (Roupioz et al. 2002; see also Sect. 12.9).

The Tg lesion rates among those ALS as shown with the help of an ODN containing this lesion at a specific position (Matray and Greenberg 1994). Interestingly, the FAPY-A lesion is readily identified as an ALS with this assay, while FAPY-G is not (Haraguchi et al. 2002). In the absence of piperidine but in the presence of phosphate buffer pH 7.5, the deglycosylation lifetime of FAPY-A is about 103 h at 37 °C; FAPY-G is about 25 times more stable (measured at 55 °C; Greenberg et al. 2001).

The *C*(1′) radical has been generated specifically within a short ss/dsODN (Tronche et al. 1998). The ratio of ALSs to SSBs was 9:1, independent of whether the radical was generated in a ss- or dsODN. The route from the *C*(1′) radical to frank SSBs is as yet unknown.

12.4.4 Strand Breaks

Concerning DNA strand breakage in the absence of O_2, the sequence of reactions resulting from the *C*(4′) radical were the first understood mechanistically (Dizdaroglu et al. 1975), and this reaction still remains the most-widely studied mechanism of DNA strand breakage. Strand breakage occurs by a heterolytic cleavage of the neighboring phosphate group, whereby cleavage at *C*(3′) strongly dominates over that at *C*(5′) [reactions (9) and (15); Beesk et al. 1979]. The heterolytic cleavage is favored over a homolytic β-cleavage due to the effective solvation of the ensuing ions (Glatthar et al. 2000).

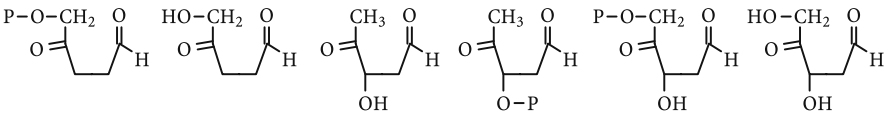


The dominant radical cation reacts with water [reactions (10) and (11)], with some preference at *C*(4′) [reaction (10)]. The radical cation has oxidizing properties and is capable of oxidizing either a neighboring G such as depicted in reaction (14) or a more distant one by hole transfer through DNA (Sect. 12.10).

Table 12.8. γ-Radiolysis of DNA in aqueous N_2O-saturated solution. *G* values (unit: 10^{-7} mol $^{-1}$) of damaged sugars resulting from an •OH-attack at *C*(4′). (Beesk et al. 1979)

Product	*G* value	First step
2,5-Dideoxypentose	0.065	Cleavage at *C*(3′)
2,3-Dideoxypentose-5-phosphato	0.07	Cleavage at *C*(3′)
2,3-Dideoxypentose	0.01	Cleavage at *C*(5′)
2,5-Dideoxypentose-3-phosphato	0.025	Cleavage at *C*(5′)
2-Deoxypentos-4-ulose	0.06	Cleavage at *C*(3′) and/or *C*(5′)
2-Deoxypentos-4-ulose-3′/5′-posphato	0.05	Cleavage at *C*(3′) and/or *C*(5′)

Analogous reactions are given by the minor radical cation [reactions (16) and (17)], and the secondary *C*(4′) radicals can eliminate the other phosphate such as reaction (18) that is shown here as an example. Reduction and oxidation reactions depicted as examples in reactions (20) and (12) lead after release of unaltered bases [reactions (13) and (21)] to a series of dideoxypentoses and *C*(4′) keto products, free and bound to DNA (Beesk et al. 1979; for the determination of these products see Chap. 13.2).



The yields of these products are given in Table 12.8. These data are fraught with a considerable error due to the analytical procedure. Moreover, it is likely that the radicals do not only undergo redox reactions as depicted. Recombination reactions with other DNA radicals present (in excess) must be considered as a potential major route. Yet, these data give a flavor as to the complexity of the reactions that follow an attack at *C*(4′).

In the presence of O_2, •OH-attack at *C*(5′) leads to the formation of 5′-aldehydes. One may envisage reactions (22) and (23) (for details of peroxyl radicals reactions see Chap. 8).

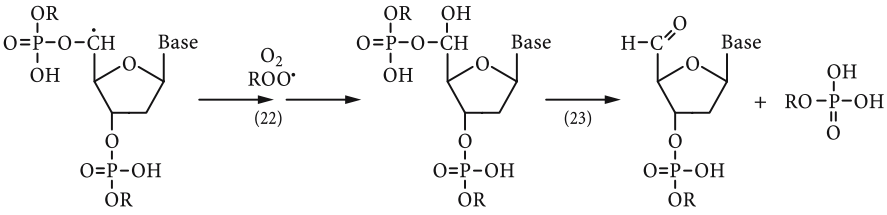


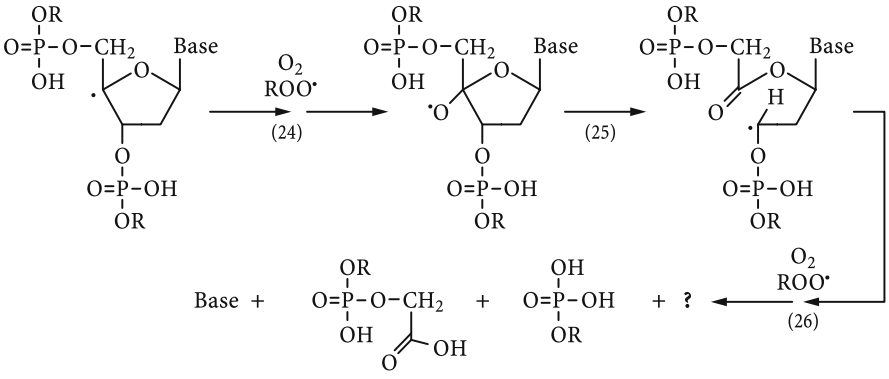
Table 12.9. Kinetic isotope effects for the formation of some DNA products connected with DNA strand breakage by •OH. (Balasubramanian et al. 1998)

Position deuterated	3′-Phosphate	3′-Phosphoglycolate	5′-Aldehyde
5′	1.67 ± 0.15		2.6 ± 0.5
4′	1.09 ± 0.55	2.1 ± 0.3	
3′	1.14 ± 0.05		
2′	1.11 ± 0.02		
1′	1.08 ± 0.03		

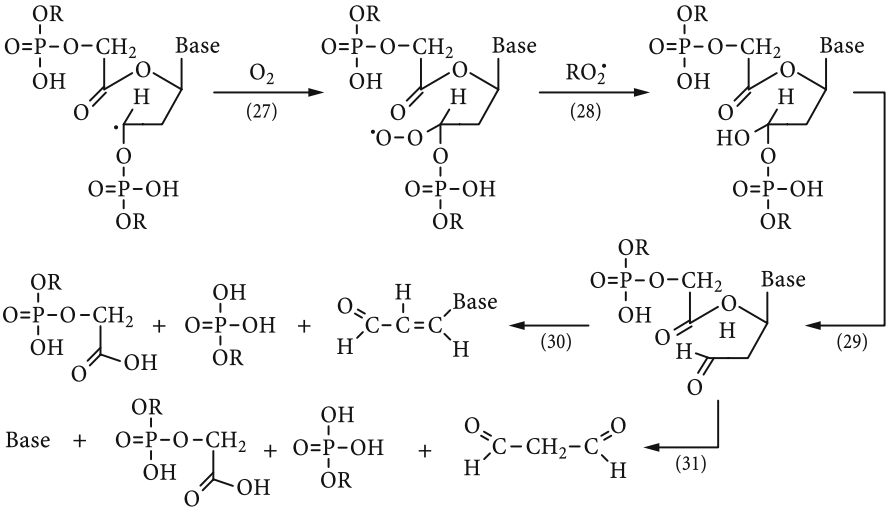
One of the most important sugar lesion is the 3′-phosphoglycolate that is typically formed in the presence of O_2 (for its excision by purified HeLa cell extracts see Winters et al. 1992). Specifically deuterated nucleoside triphosphates were used for incorporation into dsDNA by PCR (Balasubramanian et al. 1998). Hydroxyl radicals were generated by a Fenton reaction, and the yields of free 3′-phosphate end groups, 3′-phosphoglycolate and 5′-aldehyde were measured. Depending on the position of the deuteration, the yields vary with respect to a non-deuterated sample (Table 12.9).

In the original paper (Balasubramanian et al. 1998), the observed marked isotope effect has been connected with the accessibility of the various C-H groups, but we note that in its H-abstraction reactions similarly high H/D-isotope effects are observed for •OH (e.g., $k(CH_3OH)/k(CD_3OH) = 2.5$, $k(CH_3CH_2OH)/k(CD_3CD_2OH) = 1.6$ and $k((CH_3)_2CHOH)/k((CH_3)_2CDOH) = 1.5$; Anbar et al. 1966; more recently, a value of 1.96 has been reported for the EtOH system; Bonifacic et al. 2003). For the KIE to show up in an experiment, a competing reaction is required. Here, this could be, for example, an addition of •OH to the nucleobases.

There is a general agreement that its formation of the 3′-phosphoglycolate starts from the *C*(4′) radical [reactions (24)–(26)].

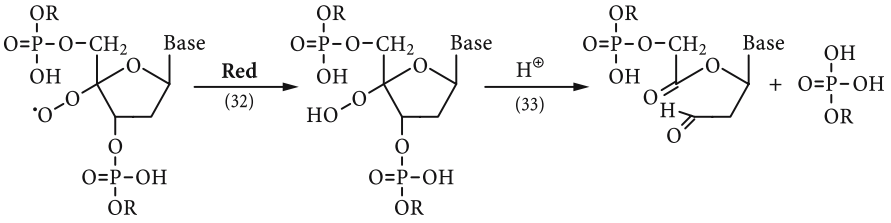


In this reaction, a 5′-phosphate end group is formed and the base is released. The products that result from the *C*(1′)−*C*(3′) fragment are not yet fully established. Malonaldehyde is a potential one. For this product to be formed, the *C*(3′) peroxyl radical has to be reduced, e.g., via the Russell mechanism (Chap. 8.8).



According to the above scheme, there is a branching into a base propenal and malonaldehyde [reactions (30) and (31)]. In the radiolysis of DNA in aqueous solution, malonaldehyde is formed (see below), but there are practically no base propenals. On the other hand, in BLM-treated DNA, base propenals dominate. If these two products have the same precursor as in the above scheme, BLM still bound to DNA must catalyze reaction (30), while without this catalysis, reaction (31) must predominate. Experiments that could elucidate this point have not yet been carried out.

An alternative, the formation of a hydroperoxide followed by an acid-catalyzed Grob rearrangement [reactions (32) and (33)] has also been suggested (Giese et al. 1995a, 1995b).



In these experiments, the *C*(4′) radical has been generated upon photolysis of the corresponding phenylselenide. The reducing agent in these experiments is as yet not ascertained. The base propenal was formed in high yields. This is in contrast to radiolytic experiments, where even with a large excess of $O_2^{\bullet -}$ as a potential

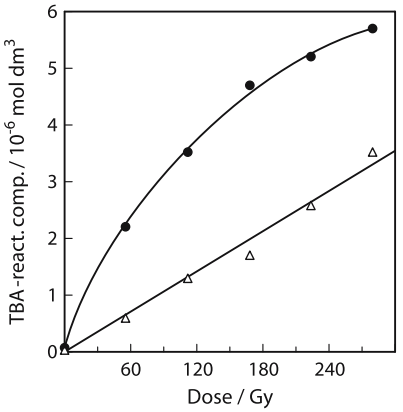
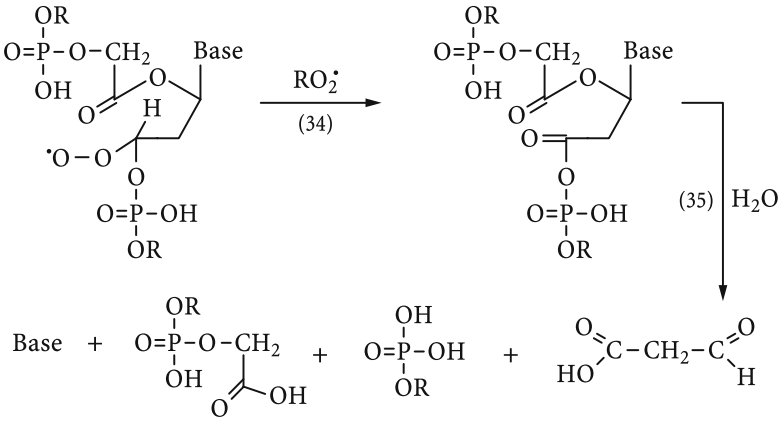


Fig. 12.11. γ-Irradiation of DNA (1×10^{-3} mol dm^{-3}) in aqueous N_2O/O_2-saturated solution. Formation of free malonaldehyde (*open triangles*) and total TBA-active material (*filled circles*, malonaldehyde plus DNA bound, in malonaldehyde equivalents as regards optical absorption) according to Rashid et al. (1999, with permission)

reductant practically no base propenals are formed (see below; for the formation of base propenals by BLM see Sect. 12.9).

In peroxyl radical reactions the formation of carbonyl functions generally dominates over a reduction (Chap. 8). This would lead to the formation malonic acid aldehyde [reactions (34) and (35)]. Being an aldehyde, it should be very easy to detect (Lipari and Swarin 1982), but the corresponding experiments seem not yet to have been carried out.



It has been known for quite some time that in the •OH reaction with DNA in the presence of O_2 lesions are formed which give rise to the formation of an intensive pink color (λ_{max} = 532 nm) upon reaction with 2-thiobarbituric acid (TBA) in acid solution (Krushinskaya and Shalnov 1967; Gutteridge 1982; Gutteridge and

Toeg 1982; Krushinskaya 1983; Kapp and Smith 1970; Ullrich and Hagen 1971; Janicek et al. 1985; Rashid et al. 1999). Nearly all the TBA-activity attributable to low-molecular-weight (DNA-free) material is due to malonaldehyde (Rashid et al. 1999), and there is no indication for the formation of base propenals which dominate the products of the BLM reaction (Sect. 12.9). Moreover, at low doses the major fraction is due to TBA-active material bound to DNA (Fig. 12.11).

Mechanistically, the formation of malonaldehyde and TBA-active DNA-bound material is not yet fully understood. While free malonaldehyde increases linearly with dose ($G = 0.1 \times 10^{-7}$ mol J^{-1} = 1.7% of •OH), the efficiency of forming TBA-active DNA-bound material markedly decreases with increasing dose, that is when the integrity of the DNA is getting lost upon damage accumulation. At one stage, $O_2^{\bullet -}$ seems to play a role, since the yield of total TBA-active material is $G = 0.3 \times 10^{-7}$ mol J^{-1} in N_2O/O_2-saturated and 0.2×10^{-7} mol J^{-1} in O_2-saturated solution (at 100 Gy). As compared to N_2O/O_2, the •OH yield is about half in O_2-saturated solutions, but the $O_2^{\bullet -}$ yield is markedly enhanced (Chap. 2.2). This could point to hydroperoxides as precursors of the TBA-active material, but details remain open. Yet, a G value of 0.2×10^{-7} mol J^{-1} (O_2-saturated) indicates that this type of lesion is not an unimportant one, about 7% of •OH.

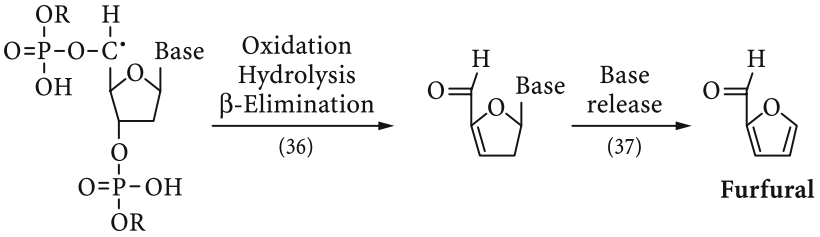
The formation of malonaldehyde induced by the Fenton reaction (Fe^{2+} plus H_2O_2 in excess) has been studied using the *N*-methylhydrazine assay (Matsufuji and Shibamoto 2004). Under such conditions, the yield of malonaldehyde is much lower (2×10^{-5} mol per mol Fe^{2+}; 1×10^{-4} when Fe^{2+} is complexed by EDTA) than in the radiolytic system discussed above. The reasons for these differences are not yet known.

The 3′-phosphoglycolate end group whose formation has been discussed above is a dominant DNA lesion formed upon γ-radiolysis as well treatment with NCS. The action of repair enzymes that might cope with this lesion has been studied (Chaudry et al. 1999).

The *C*(1′)-radical does not give rise to frank SSB as such, at least not in remarkable yields (see above), but cationic polyamines and divalent metal cations (Roginskaya et al. 2005) as well as transition metal ions such as 1,10-phenanthroline-copper ion are capable of catalyzing β-elimination processes from 2-dRL that lead to an SSB and eventually to 5-MF. These reactions are discussed in some detail below (Sect. 12.9.4). 5-MF is also produced by desferal-copper ion (Joshi et al. 1994) or oxoruthenium(IV) (Neyhart et al. 1995; Cheng et al. 1995).

The final 5-MF yield obtained after heating in the presence of polylysine as a catalyst is very high ($G = 0.5 \times 10^{-7}$ mol J^{-1} in oxygenated aqueous solution; Roginskaya et al. 2005). This is difficult to reconcile on the basis of •OH-attack at *C*(1′) as the only primary event, since H1′ is quite hidden in the minor groove (Sect. 12.2). Therefore, one is tempted to assume that a major part of the *C*(1′) damage is due to base radical attack at *C*(1′), that is due to a damage amplification reaction. Indeed, there is evidence for this type of reaction in model systems (Chap. 10.3). Because of the importance of such reactions for our understanding of free-radical-induced DNA damage this system should be investigated in more detail, and the question as to the involvement of heat-labile hydroperoxides (Chap. 11.2) should be addressed.

The minor-grove-binding artificial nuclease Mn-TMPyP when activated with $KHSO_5$ gives rise to 5-MF as well as furfural with concomitant release of unaltered nucleobases (Pratviel et al. 1991; Pitié et al. 1995). This reaction is also given by ODNs, and there is a strong variation of the 5-MF to furfural ratio in these systems indicating that structural differences determine the site of attack. While the formation of 5-MF is considered to be due to an attack at *C*(1′) (Sect. 12.9.4), furfural formation is suggested to start from an attack at *C*(5′) [reactions (36) and (37)].



Oxidation at *C*(5′) normally leads to the formation of the 5′-aldehyde, and the fact that the subsequent β-elimination and base release processes are observed may indicate that Mn-TMPyP has catalytic properties besides an oxidative power.

In the γ-radiolysis of plasmid DNA in aerobic aqueous solution, SSB formation is mainly due to the reaction of •OH with DNA. In the absence of any scavengers, an efficiency of 0.12 per •OH reacting with DNA has bee measured (Milligan et al. 1993b; 0.11 for ssDNA in the presence of N_2O/O_2 (0.15 in the presence of N_2O); Liphard et al. 1990; 0.14-0.22 at low scavenger concentrations; van Rijn et al. 1985). A variation of the concentration of added scavengers cannot be described by simple competition kinetics (Milligan et al. 1993b; Klimczak et al. 1993) but is in good agreement with a non-homogeneous kinetic model (Udovicic et al. 1991a; Mark et al. 1989). The efficiency of SSB-induction per •OH-interaction with DNA (0.32-0.44) is essentially identical for all investigated plasmids (Milligan et al. 1993b). An effect of the superhelical density is also not pronounced (Milligan et al. 1992). The non-homogeneous kinetic model can also be applied to SV40 minichromosomes where part of the DNA is protected by the histones (efficiency 0.04-0.05; Milligan et al. 1993a). The about threefold increase in the SSB induction efficiency upon going from scavenger-free aqueous DNA solutions to scavenger-containing ones is not yet fully understood. Obviously, the scavenger-free DNA solutions must always contain some impurities that could reduce the •OH-yield available for reacting with DNA, but it is difficult to visualize contaminations as high as required for factor near 2-3.

When dsDNA is γ-irradiated in dilute aqueous solution in the absence of scavengers only SSBs are formed at low doses and DSBs only become noticeable at higher doses, their yield being proportional to the square root of the dose (Hagen 1967). Under such conditions, DSBs arise from too nearby SSBs that have been created in two independent events. When •OH-scavenger are added, strand breakage is markedly reduced, but upon raising the scavenger concentration the DSB/SSB ratio increases. The formation of DSBs at high scavenger concentra-

tions has been ascribed to a single •OH hit causing a DSB and subsequent attack of the remaining sugar radical at the opposite strand causing the formation of a frank DSB (Siddiqi and Bothe 1987; see also Hempel and Mildenberger 1987). This interpretation has been challenged suggesting that these low DSB yields are due to a spur reaction involving two •OH (Ward 1988; Goodhead 1994). Modeling the clustered lesions supports the latter view (Xapsos and Pogozelsky 1996). At a scavenger capacity of 10^9 s^{-1} that approximately prevails in cells, about 40% of the DSBs have been estimated to arise from clustered lesions in the case of γ-irradiation (at high LET, this percentage is very much higher). One may also consider that not yet thermalized electrons which have been shown to be able to cause strand breakage (Chap. 4.3) may contribute in these spur reactions to DSB formation (for SSB/DSB formation upon UV excitation of solid DNA, where also low yields of 'prompt' DSBs are observed, see Sect. 12.14).

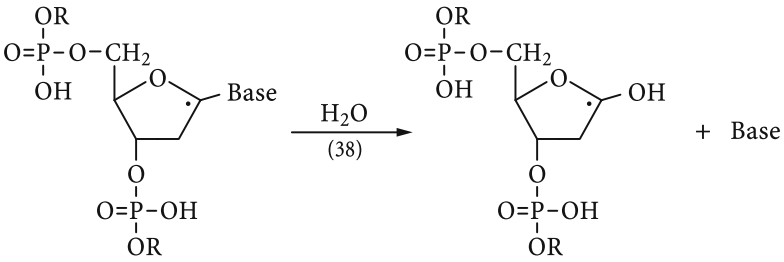
Methyl radicals react with plasmid DNA in aqueous solution with a rate constant of 8.8×10^4 dm^3 mol^{-1} s^{-1} (Milligan and Ward 1994). The nicking that is observed is due to an H-abstraction from the sugar moiety. Yet, this reaction is rather inefficient, about 0.7%, and the major reaction is most likely an addition to the nucleobases (Chap. 10). In the presence of O_2, this reaction is suppressed, but the DNA is damaged due to methylperoxyl reactions as recognized by Fpg and exoIII (Milligan et al. 1996). In the absence of O_2 and in the presence of glycerol as an •OH scavenger, the radicals derived from •OH-attack at glycerol can also nick DNA (Ayene et al. 1995). The primary glycerol-derived radicals would not be capable of undergoing this reaction, but, the secondary radicals that are formed upon water elimination (Chap. 6.9) may abstract hydrogens from the sugar moiety.

12.4.5 Base Release

The release of unaltered bases from DNA is a well-established process in free-radical-induced DNA damage in vitro as well as in vivo (Hems 1960; Ward and Kuo 1976; Richmond and Simic 1978; Richmond and Zimbrick 1975, 1981). In fact, base release was one of the first effects of ionizing radiation on DNA to be reported (Scholes and Weiss 1952). Typically, it is a biphasic (multi-phasic) reaction with some immediate base release and a marked contribution at later times, especially when the sample is heated.

For the release of an unaltered base, the sugar moiety must be damaged. In principle, the base could already be released from a radical site at the sugar moiety, i.e. on the time-scale of the lifetime of the DNA radicals. The observation of 2-dRL incorporated into DNA as a product formed upon •OH attack shows that a damage at *C*(1′) contributes to the release of an unaltered base. In the carbohydrate series, hydrolytic scission at the glycosidic linkage when this site contains a free-radical is a well-documented phenomenon, and it has been estimated that the rate of reaction must be faster than 35 s^{-1} (von Sonntag and Schuchmann 2001). As it stands, it cannot be excluded, that under certain conditions the base release from the *C*(1′) radical [reaction (38)] occurs in competition to its oxidation [reaction (2)]. In a cellular environment, there is also the reduction of DNA

radicals by GSH. The anomerization at *C*(1′) in the presence of a thiol observed on the nucleoside level (Chap. 10.3) is an indication that reaction (38) cannot be very fast.



The reactions that follow the formation of the *C*(4′) radical give also rise to products that are connected with the release of unaltered bases (see above). The decay of hydroperoxides may contribute to delayed base release when the sample is heated, as has been shown with polynucleotides (Chap. 11.2).

Thus, the time dependence of base release is most likely due to differently labile diamagnetic products that are formed side by side. Some of the damaged sites require not only considerable time for the release of unaltered bases but also heating for a prolonged time, and in cells such lesions will remain a target for the repair system.

12.5 Clustered Lesions and Damage Amplification Reactions (Tandem Lesions)

Ionizing radiation is deposited in small packages (spurs, tracks). Some of these consist of quite a number of ionizations, i.e. a number of radicals are formed in a very small volume (Chap. 2.2). When a spur (track) interacts with DNA a number of damaged sites might be formed locally (termed "locally multiply damaged sites" (LMDS); Ward 1985; Ward et al. 1990); bulky lesions or now more conveniently "clustered lesions"). The RBE of ionizing radiation increases with the ionization density of these radiations (Brenner and Ward 1992; Goodhead 1994), and this increase is interpreted as an increase in the complexity of the damage due to an increase in the number and size of clustered lesions. An example is shown in Table 12.10 (see also data in Table 12.24).

Yet this type of damage is not restricted to ionizing radiation, but certain 'radiomimetic' drugs such as BLM and NCS can similarly produce two (or more) damaged sites in close proximity (Steighner and Povirk 1990; Meschwitz et al. 1992).

First indications for such sites came from the observation that melting/reannealing kinetics differed markedly between γ-irradiated T1 DNA and the same DNA damaged by mechanical or enzymatic degradation (Martin-Bertram and Hagen 1979). It has been concluded that in addition to singly-damaged sites locally denatured sections of DNA must be formed upon γ-irradiation (see also

Table 12.10. Clustered lesions in percent of SSB induced by γ and α-particle irradiation. (Jenner et al. 2001)

System	γ-Irradiation	α-Particle irradiation
Biophysical simulations	46	93
Plasmid DNA/cell extracts	43	56
Plasmid DNA/Fpg + Nth	14	44
Plasmid DNA/Nth only	~8	26
V79 cells	11	Not determined

Table 12.11. Yields of base damage per SSB and non-DSB clustered damage per DSB, measured in plasmid DNA. (Gulston et al. 2002)

Treatment	Base damage	Non-DSB clustered damage
Nth	2.0 ± 0.3	0.8 ± 0.3
Fpg	0.9 ± 0.2	0.8 ± 0.3
Nth + Fpg	2.9 ± 0.5	1.6 ± 0.5

Martin-Bertram 1981, 1982; Martin-Bertram et al. 1983; for DNA isolated from γ-irradiated yeast cells see Andrews et al. 1984). While such lesions, detected by S1 nuclease ('S1-nuclease-sensitive sites'), are not observed with λ phage DNA γ-irradiated in aqueous solutions (Martin-Bertram et al. 1984), they become prominent when the solutions is highly scavenged (Junker et al. 1984). Under such conditions, the damage caused by the direct effect plays a role because the indirect effect is largely suppressed (Ward 1981).

Plasmid DNA when γ-irradiated in "fully scavenged" aqueous solutions (0.2 mol l^{-1} Tris) gives rise to SSBs and DSBs. The SSB yields increases upon enzymatic treatment of damaged bases with Nth and/or Fpg (Table 12.11), and so does the DSB yield (Gulston et al. 2002).

From these data, it can be seen that the effect of the two enzymes used is additive (cf. also Milligan et al. 1996). Since it cannot be expected that they can cope with all conceivable types of base damage, the ratio of base damage to SSB of 2.9 is a lower limit. Yet one has to take into account that incubation with enzymes is done at 37 °C. This induces additional 23% SSB as compared to 4 °C, where the irradiation had been carried out. Such heat-labile sites are of even higher importance for DSBs, where heating produces an extra 94%.

Clustered lesions are also apparent when DNA films are subjected to γ-radiation (Yokoya et al. 2002). Here, additional DSBs are at least twice that of prompt DSBs. In contrast, enzymatic treatment of DNA films by α-radiation does not raise the number of DSBs, and it has been suggested that in this case the complexity of the clustered damage may be greater than that formed upon γ-radiation and that the enzymes can no longer adequately work on such sites (Yokoya et al. 2003).

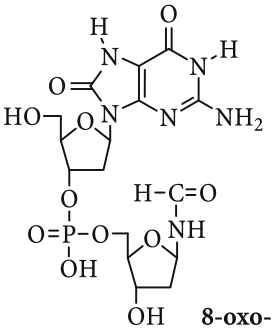
It is beyond the scope of this book to present and discuss details of the detection and repair of clustered lesions, but some papers that deal with these questions are mentioned here. For example, the action of various enzymes on clustered lesions have been investigated in some detail, such as *E. coli* EndoIII (Chaudry and Weinfeld 1995), yeast and human Ogg1 proteins (David-Cordonnier et al. 2001a,b) as well as XRS5 nuclear extract and *E. coli* Nth and Fpg proteins (David-Cordonnier et al. 2000; Gulston et al. 2002, 2004; Sutherland et al. 2000). The artificial clustered lesion, 8-oxo-G positioned on one strand and Ura on the other at 1-6 bp distance causes mutations when incorporated into wild type *E. coli* (Pearson et al. 2004). Mutation frequencies were enhanced in strains lacking the DNA glycosylases Fpg and MutY. The mutation rate decreases with an increase in the distance between the two lesions. For another clustered lesion involving 8-oxo-G and a SSB, the repair rate of the ligase III/XRCC1 complex was markedly reduced (Lomax et al. 2004). An even more complex lesion consisting of an 8-oxo-G and an 8-oxo-A on one strand and 5OHUra, 5FoUra and a one nucleotide gap on the other strand was also constructed and its repair studied (Eot-Houlier et al. 2005). Bistranded APs in close proximity are clustered lesions that could turn into a DSB upon the action of repair enzymes. For this reason the action of human Ape and *E. coli* Exo III on a variety of such lesions have been investigated in some detail (Chaudry and Weinfeld 1997).

12.5.1 Tandem Lesions

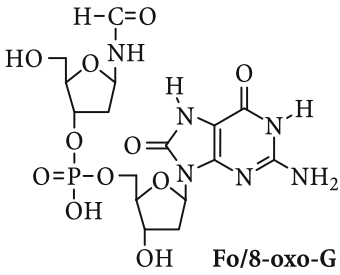
The tandem lesion, such as two adjacent damaged bases at the same strand, is a special case of a clustered lesion. Mechanistically they are very interesting, since they are induced by a single radical, that is, they are due to a damage amplification reaction. Mechanistic aspects are discussed in Chapters 10 and 11.2. Since clustered lesions constitute a severer damage than a single lesion, it is conceivable that 'repair' of DNA radicals by thiols (Sect. 12.12) that reduces the severity of damage, especially in the presence of O_2, also interferes with the formation of tandem lesions. To our knowledge, this has not yet been investigated. The longevity of DNA peroxyl radicals allow intramolecular reactions to occur that would be very much less pronounced on the nucleotide level. This may be concluded from the observation that $^{\bullet}OH$-induced O_2-uptake is noticeably higher in DNA than in an equivalent mixture of nucleotides (Isildar et al. 1982).

One of these tandem lesions in DNA consist of a Fo (precursor: T or C) next to an 8-oxo-G lesion (Box et al. 2000; Maccubbin et al. 2000; Bourdat et al. 2000; Cadet et al. 2002). Within DNA, two types of this lesion are expected, the 8-oxo-G/Fo and the Fo/8-oxo-G lesion. They are formed in considerably different

yields, 0.001 and 0.013 × 10^{-7} mol J^{-1}, respectively (Bourdat et al. 2000; Cadet et al. 2002).



8-oxo-G/Fo

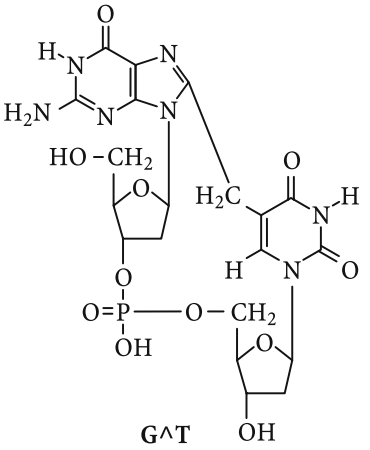


Fo/8-oxo-G

Under the same conditions, the total 8-oxo-G yield is 0.13 × 10^{-7} mol J^{-1}. Thus the tandem lesions contribute about 10% of the total 8-oxo-G yield in DNA. Using another approach to isolate these lesions gave G(Fo/8-oxo-G) = 0.12 × 10^{-7} mol J^{-1}, i.e. a value which is an order of magnitude higher (Maccubbin et al. 2000). In this study, this lesion was also observed in the absence of O_2, albeit with a much lower yield. Since Fo can only be formed in the presence of O_2, one must conclude that these samples were not fully deoxygenated. When the effect of EDTA-Fe^{2+} was studied, it was observed that the yield of these lesions increased with time (Bourdat et al. 2000). It was not realized that EDTA-Fe^{2+} readily autoxidizes in the presence of O_2 and that this reaction is connected with a concomitant formation of $^{\bullet}OH$ (Yurkova et al. 1999; Chap. 2.5). Mechanistic details seem now to be reasonably well established and have been discussed in Chapter 10.6. The mechanism originally suggested (Bourdat et al. 2000), namely that the T-derived peroxyl radical oxidizes a neighboring G by one-electron oxidation, is very unlikely considering that the reduction potential of this kind of peroxyl radicals is only +0.8 V (Chap. 8.3) and the reduction potential of G is near +1.3 V (see above) rendering this reaction markedly endothermic. This point has been raised here, since oxidation of G observed in systems that involve peroxyl radicals sometimes make use of the ET concept.

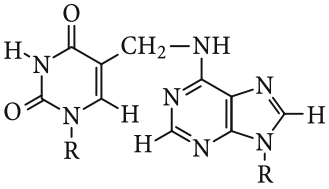
The action of nuclease P1 on the 8-oxo-G/Fo lesion has been studied on the model level (Maccubin et al. 1992).

In the absence of O_2, the G^T lesion is formed in DNA that has been γ-irradiated in aqueous solution (Box et al. 2000; for model systems see Chap. 10.5).



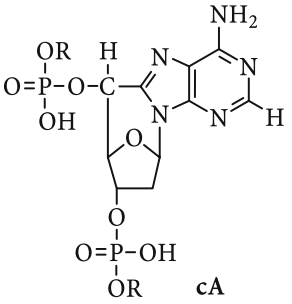
In DNA, there should also be the corresponding T^G, A^T and T^A formed, but these have not been detected as yet. The T^G lesion is most likely less abundant than the G^T lesion, since in a GTG-containing 15-mer the G^T lesion strongly dominates over the T^G lesion (in a ATA-containing 15-mer A^T dominates over T^A; Bellon et al. 2002).

A cross-link between T at one strand and A at the opposite one is induced by the allylic T radical (Hong and Greenberg 2005).

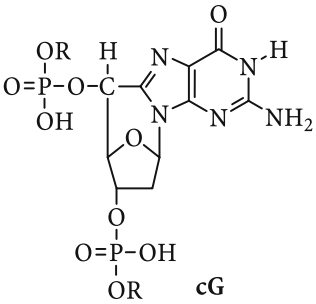


This cross-link is observed in the absence of O_2 but also in its presence, and future work will be required to substantiate mechanistic details of this most interesting lesion.

cA and cG were the first tandem lesions that have been detected (Chap. 10.5), and ever since these interesting lesions have found considerable attention. They are also detected in γ-irradiated DNA (Brooks et al. 2000; Dirksen et al. 1988; Fuciarelli et al. 1985; Haromy et al. 1980; Raleigh and Blackburn 1978; Randerath et al. 2001; Romieu et al. 1999b). Upon •OH-attack in aqueous solution the *R*/*S* ratio of cG is 0.3 (Jaruga et al. 2002; 0.24 - Birincioglu et al. 2003). This ratio changes to 0.5 when DNA is treated with one-electron reduced tirapazamine (Birincioglu et al. 2003). For cA this ratio is 3 for •OH and 77 for one-electron reduced tirapazamine (Sect. 12.9.6).

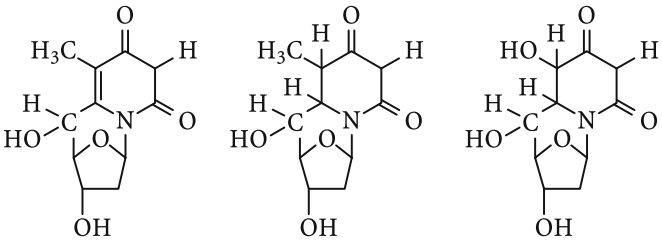


cA

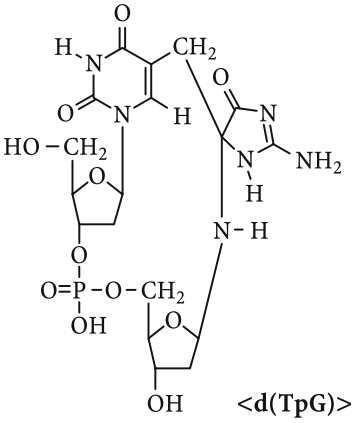


cG

On the model level, a cyclization reaction involving the *C*(5′) radical and *C*(6) have been observed with Thd and dCyd (Chaps 10.10 and 10.5), but such products were as yet not detected in DNA.



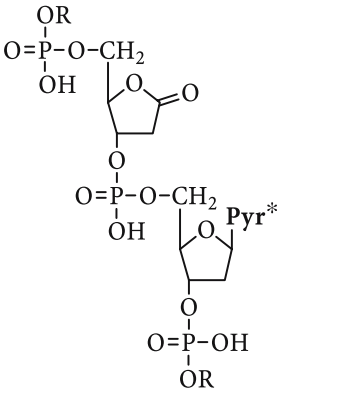
This also holds for the <TpG> lesion that is formed upon photooxidation in the presence of O_2 (Chap. 10.11).



<d(TpG)>

Here again, the allylic T radical has added to a neighboring G, but subsequent addition of O_2 must have degraded the G moitey. Since in the nucleus the O_2 concentration is rather low, this type of reaction stands a good chance to play a role.

Another damage amplification reaction has been observed with a dinucleotide model which allowed to generate a Thy-5-yl radical specifically. In the presence of O_2, it causes the formation of the 2-dRL lesion at the adjacent nucleotide unit (Chap. 10.3). Based on this, •OH-addition to *C*(6) of T and C could give rise to this tandem lesion also in DNA (as yet not detected).



Pyr* = damaged T or C

It is as not known to what extent the observed formation of 2-dRL in DNA is due to such a tandem lesion (see also Sect. 12.4.4; note that H1′ is hidden in the minor groove (Sect. 12.2) and not very likely to be attacked by freely-diffusing reactive radicals such as •OH).

12.6 DNA-DNA Cross-Links and DNA-Protein Cross-Links

12.6.1 DNA/DNA Cross-Links

Several types of DNA-DNA cross-links may be envisaged. Cross-linking of two DNA molecules that lead to an increase in the molecular weight is original definition of a DNA/DNA cross-link. As cellular systems are concerned, this is a rather unlikely event. In a mammalian cell, for example, a cross-link between two chromosomes would have to occur. Thus, this type of DNA damage is restricted to artificial systems such as irradiation of DNA in the solid state (Lücke-Huhle et al. 1970). Interestingly, absence or presence of O_2 has practically no effect on their yields under such conditions, while SSB and DSB yields are doubled in its presence. Data obtained with artificial polymers in aqueous solution suggest that recombination of two DNA radicals situated at the same DNA should be a much more likely process than a recombination of two radicals at different DNA macromolecules (Chap. 9.6). This would form a kind of loop and would not be connected with an increase in molecular weight (for a tandem lesion connecting two opposite bases see Sect. 12.5.1; for interstrand cross-links by enediyne

antitumor antibiotics, see Sect. 12.9.3). The limited flexibility of DNA may favor rather large loop sizes. In dsDNA, this type of process could, in principle, connect not only two sites of the same strand but also two opposite sites (at a distance). Some of the tandem lesions mentioned above (Sect. 12.5.1) are a kind of intrastrand mini-cross-link.

In cells, DNA is always associated with proteins, in eukaryotic cells with the nucleoproteins. This 'packaging' will further reduce the mobility of DNA segments that may carry a free radical, and their recombination with concomitant cross-linking is further slowed down. This will allow chemical repair processes such as H-donation by GSH to take place (Sect. 12.12).

12.6.2 DNA–Protein Cross-Links

In principle, DNA-protein cross-links may be formed by the recombination of a DNA radical with a protein radical. This trivial reaction requires that the two radicals are formed independently and are formed in such a sufficient proximity that they react with one another rather than undergo other reactions such as reactions with O_2 or GSH. Radicals in close proximity are formed in the spurs of ionizing radiation, and under such conditions DNA–histone cross-linking may occur in the cellular environment where only those $^{\bullet}OH$ radicals that are formed in the very neighborhood of DNA contribute to DNA damage. Yet, biologically-active DNA is inactivated by the $^{\bullet}OH$-adduct of phenylalanine (by addition?; de Jong et al. 1972), and DNA–protein cross-links are also observed when DNA protein mixtures are subjected to ionizing radiation in aqueous solution (Schüssler and Jung 1989; Schuessler et al. 1997; Distel et al. 2002). This reaction can also be triggered by secondary radicals like the hydroxyethyl radical (Schuessler et al. 1992). Altogether, this cross-linking must be due to a complex sequence of reactions whose details are as yet not known.

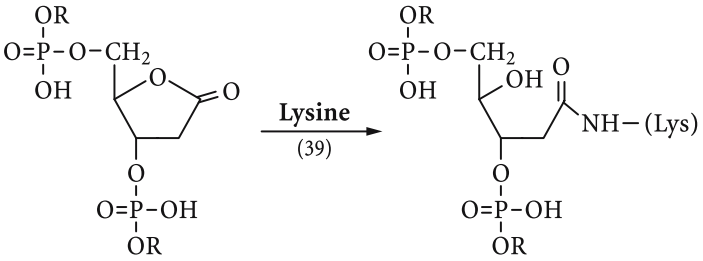
Mechanistically, much more interesting are DNA-protein cross-links that are generated by a single radical. As has been shown by the flash-quench technique, the G-derived radical is a good candidate (Nguyen et al. 2000). This technique uses a ruthenium-based intercalator which after photoexcitation can be oxidized by a quencher such as a Co(III) complex. The resulting intercalator in its higher oxidation state can now oxidize G (G-free ODNs do not show this cross-linking reaction). The deprotonated $G^{\bullet+}$; that is, $G^{\bullet}$, has been suggested to undergo the observed cross-linking reaction. As the nature of this cross-link is concerned, the authors refer to studies that showed that basic amino acids such as serine, lysine and threonine can covalently link to the radical formed by the reaction of photoexcited benzophenone with dGuo and a dGuo derivative (Morin and Cadet 1994, 1995a, b). Proteins attached to DNA mediate hole transfer through DNA (Wagenknecht et al. 2001), and this kind of process may lead to a DNA-protein cross-link, for example, via tyrosine (Wagenknecht et al. 2000).

Besides DNA strand breaks, DNA-protein cross-links are also formed upon photodynamic action of chloroalbumine phthalocyanine (Ramakrishnan et al. 1988) and by a large variety of other photosensitizers (Villanueva et al. 1993).

The involvement of singlet O_2 ($O_2{}^1\Delta_g$) has been ascertained by showing that the rate of cross-linking increases when the solvent water is replaced by D_2O. G has the by far highest singlet O_2 rate constant among the DNA constituents (k(DNA) = 7×10^5; k(dGMP) = 5×10^6; k(dAMP) = 7.5×10^4; k(CMP) = 2.4×10^4; k(TMP) = 1.5×10^5 dm^3 mol^{-1} s^{-1}; Wilkinson et al. 1995), and a damaged G may be a responsible for the cross-linking reaction.

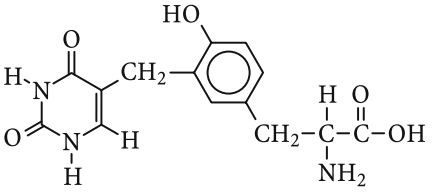
Peroxidized proteins (cf. Gebicki et al. 2002; Simpson et al. 1992; Gebicki and Gebicki 1993; Gieseg et al. 2000; for a review see Dean et al. 1997) in their reaction with DNA yield DNA-protein cross-links (Gebicki 1997; Gebicki and Gebicki 1999; Luxford et al. 1999). Complexation of transition metal ions by, e.g., desferal reduces their yield but does but fully prevent cross-linking. DNA strand breakage was not observed under these conditions. Mechanistically, it is not yet understood, how protein hydroperoxides interact with DNA.

An important contributor to DNA-protein cross-links may be the reaction of DNA products with proteins. A case in point is the cross-linking of the relatively abundant DNA damage 2-dRL with a lysine moiety [reaction (39); Hashimoto et al., 2001].



This type of reaction inhibits the repair by endonuclease III from *E. coli*, and Lys-120 is responsible for this cross-linking reaction. Under the conditions of these experiments, the cross-linking efficiency is 20%. It is expected that such cross-linking reactions are more general and that more examples will be detected.

DNA–protein cross-links were observed in mammalian cells upon ionizing radiation (150 per Gy and cell with a considerable background level of ~6×10^3 per cell; Ramakrishnan et al. 2003) but also upon addition of Fe^{2+} (but not with Cu^{2+}) to the culture medium (Altman et al. 1995). The Thy-tyrosine cross-link shown below has been identified by GC/MS of enzyme-digested material, but mechanistic details as to the formation of this interesting cross-link are as yet not known.



DNA-protein cross-links are quite abundant DNA lesions in mammalian cells, but per se they are not lethal and can be enzymatically repaired (Chiu et al. 1989, 1990; Oleinick et al. 1986; Chiou et al. 1984).

12.7 5-Halouracil-Substituted DNA

5-Bromo- or 5-iodouracil-substituted DNA has been investigated in some detail for studying free-radical-induced strand breakage (ALSs; for reviews see Hutchinson and Köhnlein 1980; Hutchinson 1987). Moreover, BudR-labeling CHO cells induces quite significantly sister chromatid exchanges (SCE) upon treatment with UV and ionizing radiation (Wojcik et al. 1999, 2003; Bruckmann et al. 1999). The 5-uracilyl radical formed by either photolysis or ionizing radiation is capable of abstracting a hydrogen from a neighboring sugar moiety (Chap. 10.7). This leads to an ALS and subsequently to an SSB (Krasin and Hutchinson 1978a; Hewitt and Marburger 1975), but DSBs are also observed in a single photochemical event in BudR-labeled *E. coli* cells (Krasin and Hutchinson 1978b). This is one of the many indications that in dsODNs and in DNA the photochemical reaction is much more complex than in the simple model systems that were described in Chapter 10.7. The quantum yield of Ura formation of 5BrUra in dilute aqueous solution in the presence of low concentrations of H-donors is more than an order of magnitude lower than that of SSB formation in 5BrUra-substituted DNA (Campbell et al. 1974). This has been attributed to a prevention of cage recombination by scavenging caged radicals. However, there is a strong influence of neighboring bases (Murray and Martin 1989), and this is not compatible with a simple homolytic cleavage of the C-Hal bond as in the photolysis of the halouracils without such neighboring effects.

From an extended study on the sequence selectivity of UV-induced cleavage of dsODNs (Table 12.12) it has been concluded that an ET to neighboring bases must occur [reaction (40)] followed by a subsequent competition between electron backdonation [reaction (41)], decay of the 5BrUra radical anion [reaction (42)] and hole transfer [reaction (43); Chen et al. 2000.

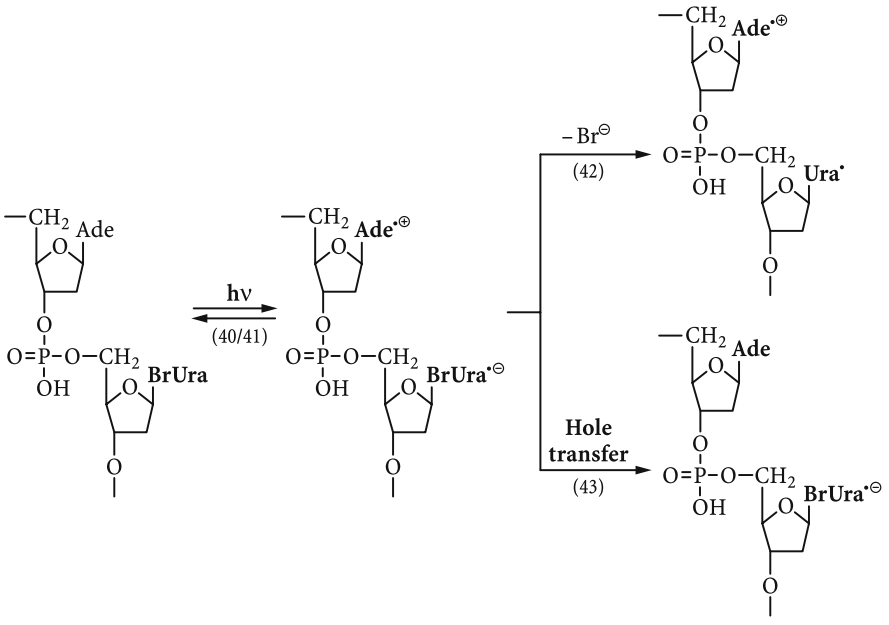
Table 12.12. Sequence selectivity for UV-radiation (302 nm) induced direct strand scission in ODNs containing two 5BrUra sites. (Chen et al. 2000)

11 21

5'-d(CGCATATGGC**X**BuGCTATAGCABuGCCGCATATG
d(GCGTATACCG**Y** A CGATATCGT A CGGCGTATAC

Duplex	X•Y	(Cleavage ratio $A_{21}/X_{11})_{observed}$	(Cleavage ratio $A_{21}/X_{11})_{normalized}$
I	A•T	1.7 ± 0.1	
II	G•C	36.4 ± 1.6	21.4
III	C•G	5.8 ± 0.1	3.6
IV	U•A	2.4 ± 0.2	1.4
V	I•C	4.3 ± 1.4	2.5
VI	AA•T	4.7 ± 0.9	2.8

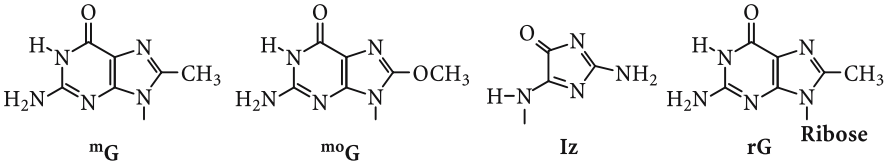
The normalized value is the observed value divided by the observed value of I, i.e., corrected for the natural bias in I



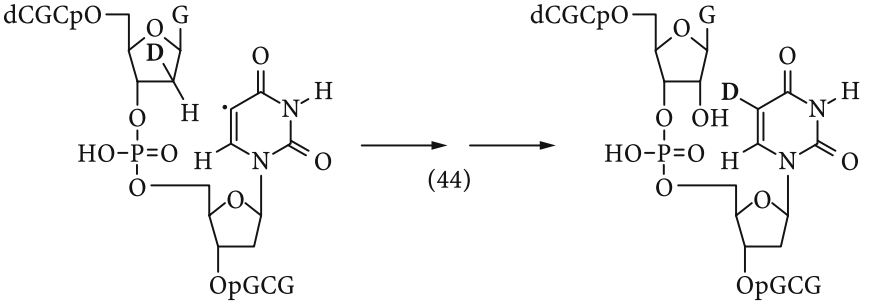
To study structural effects in the ET reactions, 5BrUra has been incorporated in dsODNs that can be made to form either B-DNA or Z-DNA type conformations (Tashiro and Sugiyama 2003). The ODN1/ODN2 in B-DNA type conformation did not show any photoreactivity. Yet, in 2 mol dm^{-3} NaCl, where this dsODN assumes a Z-DNA type conformation afforded rG in 35% and Iz in 33% yield based on ODN1 consumed. Even more striking are the results with the ODN1/ODN3 couple. In the B-DNA conformation, it is again not photoreactive, while in the Z-DNA conformation it yields 80% rG and 75% Iz.

ODN1	5′-d($C_1G_2C_3$**G_4**^{Br}**U_5**$G_6C_7G_8$)-3′	ODN1	5′-d(CGCG^{Br}UGCG)-3′
ODN2	3′-d($G_{16}C_{15}{}^{m}G_{14}C_{13}A_{12}$**$C_{11}$**^{m}**$G_{10}$**$C_9$)-5′	ODN3	3′-d(GC^{m}GCAC^{mo}GC)-5′

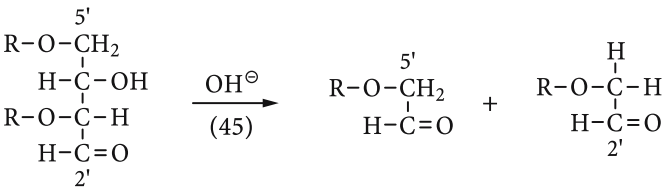
In B-form ODN1/ODN2, ET occurs from a neighboring G to the 5BrUra moiety, but efficient electron back-donation renders this reaction without consequence. In Z-form ODN1/ODN2, however, G_4–$^{Br}U_5$–C_{11}–$^{m}G_{10}$ forms a unique four-base π-stack cluster. This allows the hole to migrate to and be trapped at the $^{m}G_{10}$ site which has a lower reduction potential than the G_4 site. The $^{mo}G_{10}$ site in the ODN1/ODN3 couple can trap the hole even more effectively, and thus the reaction becomes more efficient. As a consequence, the uracilyl radical is formed and abstracts an H2′ of the G_4 site which in subsequent reactions involving O_2 is transformed into rG. The oxidized $^{m}G_{10}$ ($^{mo}G_{10}$) shows up as the Iz lesion.



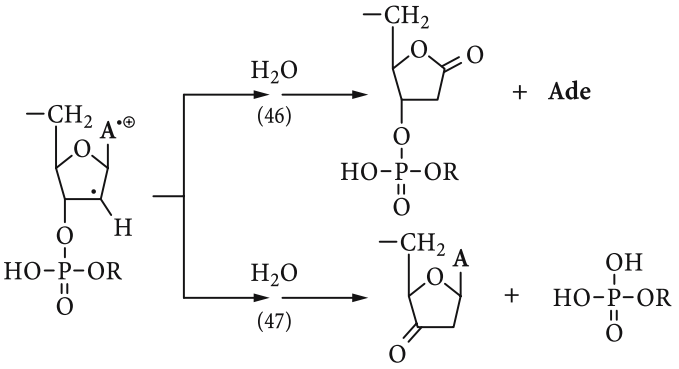
With adequately tailored short Z-form DNA duplexes, H-transfer occurs by intrastrand β-H2′-abstraction [reaction (44); Kawai and Saito 1999; Kawai et al. 2000a], while competitive H1′- and α-H2′-abstractions occurs in B-form DNA (Sugiyama et al. 1990, 1993, 1996). Predominant H1′-abstraction is observed in a DNA-RNA hybrid (Sugiyama et al. 1997).



Upon attack at *C*(2′) and in the presence of O_2, the sugar moiety is mainly modified into an erythrose unit (Sugiyama et al. 1993) which is an alkali-labile site and undergoes a retroaldol reaction after deprotonation of H4′ [reaction (45)], but a Z-form type structure favors *C*(2′)-hydroxylation.



Based on kinetic isotope effects, it has been suggested that the observed formation of the 2dRL lesion in the anaerobic photolysis of 5BrUra-containing duplex DNA may not be caused by H1′-abstraction (Cook and Greenberg 1996). It has been proposed that in the excited state ET occurs from a neighboring A to 5BrUra [cf. reaction (40)]. Oxidation of the *C*(2′)-radical (formed by H-abstraction by the 5-uracilyl radical) by $A^{\bullet+}$ and subsequent 1,2-hydride shifts have been suggested to lead to the observed products [reactions (46) and (47)].



When BrdUra- or IdUrd-substituted DNA is in contact with a protein, cross-linking between DNA and the protein is observed upon UV-irradiation (Ogata and Gilbert 1977; Babkina et al. 2002; Hicke et al. 1994). The chemistry of this interesting reaction has not yet been elucidated. Interstrand DNA cross-links are among the most potent inducers of sister chromatid exchanges (SCE), and hence the high efficiency of SCE formation in the photolysis BrdUra-labeled DNA has been tentatively attributed to cross-linking (Wojcik et al. 2003), and in the meantime this hypothesis has been experimentally substantiated (Wojcik et al. 2005).

In the case of sensitization of halouracil-containing DNA with ionizing radiation, attention has been drawn to potential contributions of resonant electron capture of low-energy electrons (Abdoul-Carine et al. 2001). The reaction of e_{aq}^- with 8-Br-dGuo (Chap. 10.7) incorporated into DNA leads to a conversion of 8-Br-G into G in high yields with Z-form DNA being more effective than B-form DNA (Kimura et al. 2004).

12.8 Hydrogen Peroxide and Organic Hydroperoxides

The efficiency of H_2O_2 to inactivate living cells is rather low compared to other oxidizing agents such as ozone, and relatively high concentrations combined with long exposure times are required (e.g., Prise et al. 1989; Toledo et al. 1998; Thacker 1975; Swartling and Lindgren 1997; the fact that spores are quite resistant against H_2O_2 is a major concern in the food industry; Ito et al. 1973; Stevenson and Shafer 1983, where H_2O_2 is an important disinfectant; for reviews on the mechanisms of spore resistance, see Setlow 1995; Marquis et al. 1994). As an uncharged and low-molecular-weight compound, H_2O_2 can diffuse readily through membranes. Thus, there is no major barrier to reach the DNA. It is now generally agreed that DNA damage is due to a Fenton-type reaction (for details of Fenton-type reactions see Chap. 2.5). Evidence for this has been obtained by the addition of DMSO as $^{\bullet}OH$-scavenger which reduces SSB formation (Ward et al. 1985) and chromosomal aberrations (Oya et al. 1986) in vivo as well as base damage in vitro (Blakely et al. 1990). Moreover, when the DNA was freed from transition metal ions by exhaustive dialysis with a transition metal ion chelator an effect of H_2O_2 was no longer observed. Although this experiment shows that transition metal ions must be involved, it does not discriminate between iron or copper as the relevant agent, and there is an ongoing discussion as to what extent these two transition metal ions contribute to DNA damage in cells (Barbouti et al. 2001; Bar-Or and Winkler 2002; Galaris et al. 2002). Mitochondrial DNA shows a 10-15 times higher steady-state level of damaged bases, and this has, in part, been attributed to the higher iron levels in mitochondrial as compared to nuclear DNA (Eaton and Qian 2002).

In mammalian cells, the number of SSBs induced at equitoxic doses is much greater for H_2O_2 than for X-rays (Bradley and Erickson 1981). HeLa cells exposed to 1×10^{-4} mol dm^{-3} H_2O_2 at 0-1 °C results in the same number of SSBs as a dose of 300 Gy (Szmigiero and Studzian 1988). Based on a value of 1000 SSBs per cell per Gy (Elkind 1979), a somewhat higher value of 6000 Gy equivalent was estimated from extrapolated data (Ward et al. 1985). In Chinese hamster V79 cells, such lesions were already detected upon incubation with 1×10^{-5} mol dm^{-3} H_2O_2 at 4 °C for 20 min (Prise et al. 1989). In addition, damaged bases are formed as has been shown in in vitro (Demple and Linn 1982; Blakely et al. 1990) and in in vivo studies (Dizdaroglu et al. 1991).

The ratios of two typical A and G products are compiled in Table 12.13 for γ-radiolysis in N_2O/O_2-saturated solutions which produces 90% $^{\bullet}OH$ and 10% $O_2^{\bullet-}$ and for H_2O_2. If in the reaction of DNA-bound transition metal ions with H_2O_2 $^{\bullet}OH$ would be the only agent that is responsible for the observed damage one would expect that these product ratios should be identical, but they are markedly different (in order to avoid potential uncertainties, data from the same group where chosen).

Since in both cases the primary damage is set by $^{\bullet}OH$, these differences can be explained if the transition metal ion which must be still near the damaged site modifies the damage prior to a reaction of oxygen. Alternatively, H_2O_2 which was present at rather high concentrations in these experiments (0.1 to 0.4 mol

Table 12.13. Product ratios of typical purine products formed by the reaction of •OH (γ-radiolysis) and by H_2O_2 in O_2-containing solution

Product ratio	•OH	H_2O_2	Reference
8-oxo-A/FAPY-A	2.7	0.06	Fuciarelli et al. (1990); Blakely et al. (1990)
8-oxo-G/FAPY-G	12.5	3.4	Fuciarelli et al. (1990); Blakely et al. (1990)
8-oxo-G/Z	1.7	4.1	Douki and Cadet (1996)
8-oxo-G/8-oxo-A	21	4.2	Douki and Cadet (1996)

dm^{-3}) might have interfered at this stage (for the reaction of radicals with H_2O_2 see Chap. 6.7). It may be worth pointing out that marked differences in product ratios was also observed with deoxyribonucleosides not only when γ-irradiation was compared with Fenton-systems, but also among different iron-based Fenton-systems (Murata-Kamiya et al. 1998).

For the Fenton reaction to proceed, the transition metal ion must be in its low oxidation state. For example, iron must be present as Fe^{2+} [reaction (48)].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (48)$$

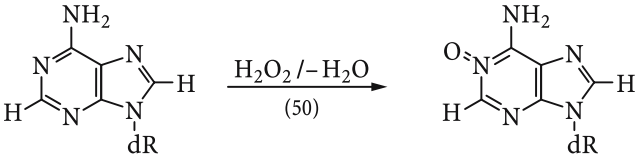
When all the Fe^{2+} is consumed (the intracellular labile iron pool is around micromolar; Epsztejn et al. 1997), the reaction should come to a standstill. In the cellular systems that have been investigated, this seems not to be the case, and it has been postulated that Fe^{3+} (possibly complexed to DNA) is reduced by H_2O_2 in a slow reaction [reaction (49); Ward et al. 1985].

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+ \quad (49)$$

In vitro, reaction (49) is very slow, and a value of 0.27 $dm^3\ mol^{-1}\ s^{-1}$ has been suggested (Henle et al. 1996). This value is essential for the understanding of the Fenton reaction and requires further substantiation. In cells, the transition metal ions in their high oxidation states may become reduced by cellular reducing agents such as ascorbate (cf. the Udenfriend variation of the Fenton reaction, Chap. 2.5), or by any other metabolically generated strong reductant. Spores which lack any metabolism are only effectively inactivated by H_2O_2 at high temperatures. The reactions that occur under such conditions have not yet been elucidated.

With Cu^{2+}, H_2O_2 and ascorbate, a Fenton-type reaction is initiated. In the presence of DNA, the copper is bound to DNA and an addition of •OH scavengers such as DMSO suppresses this reaction only marginally as shown with a polylysine-bound fluorescent •OH probe (Makrigiorgos 1999).

Besides inducing a Fenton-type (i.e., free-radical) chemistry, H_2O_2 (at high concentrations) can oxidize nucleobases, and in the case of Ade and its derivatives the formation of the N^7-oxide has been reported (Rhaese 1968), and further reactions seem to occur as well. This lesion, now attributed to the N^1-oxide [reaction (50)], has been detected by the ^{32}P-postlabelling technique (Mouret et al. 1990) and polyclonal antibodies have been raised to detect this lesion in oxidized DNA (Signorini et al. 1998).



In fact, adenine-N^1-oxide is an important DNA lesion, when *Proteous mirabilis* cells were exposed to H_2O_2 (Cadet et al. 1989).

The action by H_2O_2 produces per lethal event five times fewer DSBs than X-rays (Prise et al. 1989). In contrast to ionizing radiation, H_2O_2 does not create DNA-protein cross-links (Bradley and Erickson 1981) and causes very few if any mutations (Thacker 1975; Thacker and Parker 1976; Bradley and Erickson 1981).

Autoxidation of lipids leads to the formation of hydroperoxides, and such products have been considered to be capable of inducing cancer (Ames 1983; Ames and Shigenaga 1992). With supercoiled pBR322 plasmid DNA and 13-L_5-hydroperoxy-*cis*-9,*trans*-11-octadienonic acid as a model, SSBs and DSBs are formed at G sites (Inouye 1984). Mechanistic details, e.g., the potential involvement of transition metal ions, have not been investigated. The addition of Fe^{2+} to the culture medium increases DNA base damage in mammalian cells markedly above control level at least for some of the lesions studied (Zastawny et al. 1995). Yet, there is no linear correlation between the concentration of added Fe^{2+} and base damage yields. In these cells, the cell membrane permeability increases upon addition of Fe^{2+} as does the lipid peroxidation. Addition of DMSO cannot reduce the damage, and it has been concluded that induction of DNA damage must be a site-specific reaction involving DNA-bound Fe^{2+}.

It is noteworthy that in the presence of H_2O_2 not only iron and copper, but also nickel and cobalt induce DNA damage via a Fenton-type reaction (Nackerdien et al. 1991).

12.9 DNA Cleaving Drugs

12.9.1 General Remarks

There are a number of drugs that intercalate very specifically into DNA with one part of the molecule in a way that brings another part that is capable of creating free radicals into a position to abstract an H-atom from the sugar moiety, again

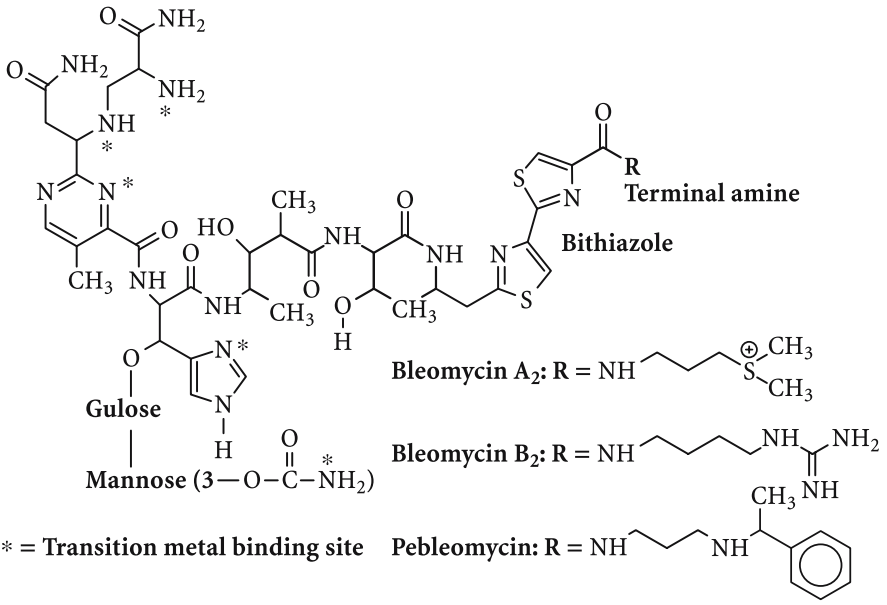


Fig. 12.12. The structure of bleomycins. (Adapted from Burger 1998)

very specifically (Pratviel et al. 1995). As a consequence, SSBs may be created or at least ALSs that transform into an SSB upon alkali treatment, but the nucleobases are not damaged or only to a very minor extent. Such DNA cleavers are often called 'radiomimetic drugs', but this term is misleading for most of them, since there are no free •OH or any other reactive intermediates formed that are typical for ionizing radiation. The literature on this topic is vast, and it is not possible to quote it here adequately. Only the major mechanistic aspects can be dealt with here, and for further details the reader is referred to reviews on this topic (bleomycin: Hecht 1979; Povirk 1983; Stubbe and Kozarich 1987; Dedon and Goldberg 1992; Natrajan and Hecht 1994; Burger and Drlica 1996; Stubbe et al. 1996; Burger 1998, 2000; neocarzinostatin: Dedon and Goldberg 1992; calicheamicin: Dedon and Goldberg 1992) and the references quoted in the papers that are mentioned below.

12.9.2 Bleomycin

The bithiazole group of the bleomycins, BLMs (Fig. 12.12), intercalates into the minor groove of DNA (Povirk et al. 1979). The transition metal ions are co-ordinated by various nitrogens as indicated by asterisks (for a review of the coordination of BLMs, see Dabrowiak 1980; for more recent data, see Chikira et al. 2000; Kemsley et al. 2003).

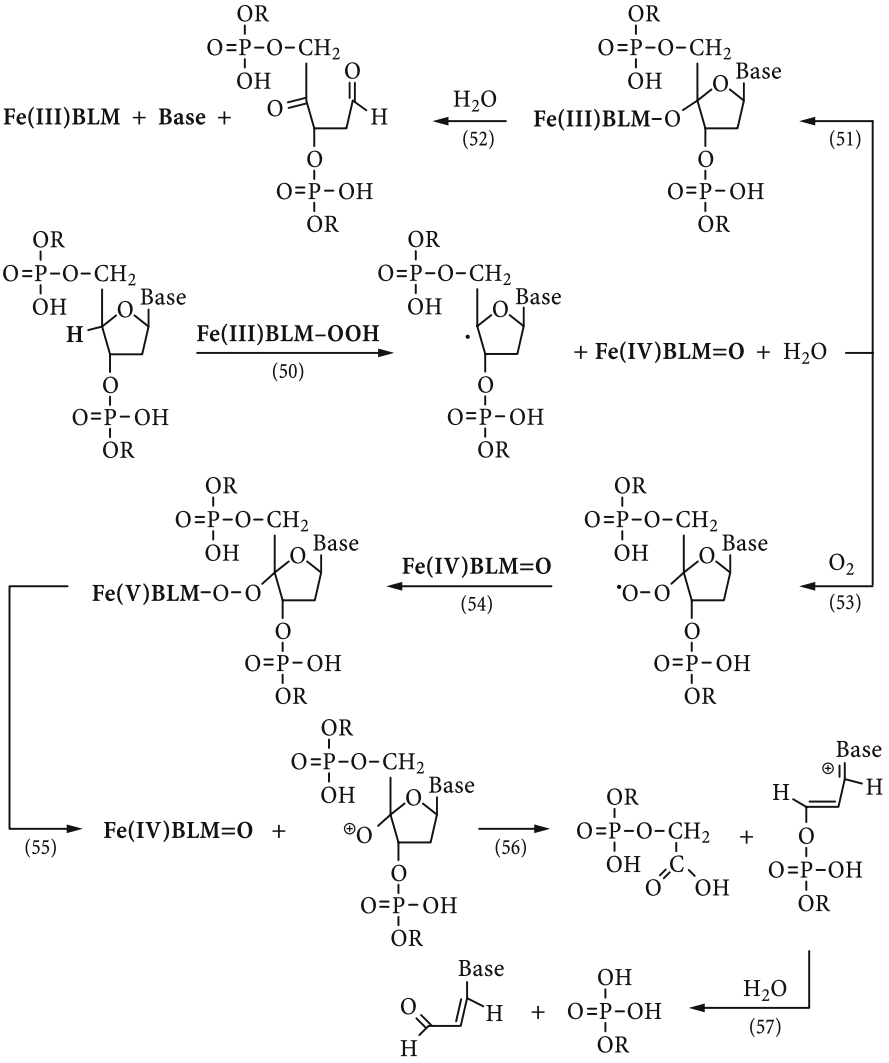
The DNA-cleaving BLM contains iron and is supplied as Fe(II)BLM. Yet, Mn(II)BLM activated by H_2O_2 may also cleave DNA (Burger et al. 1984). In solution, Fe(II)BLM binds O_2 (Burger et al. 1979, 1983), and this adduct is activated by a reducing agent such as Fe^{2+} (Burger et al. 1980) and seems even further stimulated by $O_2^{\bullet -}$ (Ishida and Takahashi 1975). The 'activated BLM' is a ferric hydroperoxide complex Fe(III)BLMOOH (Sam et al. 1994). It may also be formed by reacting Fe(III)BLM with H_2O_2 (Burger et al. 1982b, 1983). Its position within DNA is such that its active site reacts nearly exclusively by abstracting H4′ (Wu et al. 1985a; Absalon et al. 1992). One may envisage as potential routes: (a) homolytic cleavage into Fe(IV)BLM=O and $^{\bullet}$OH, (b) heterolytic cleavage into Fe(V)BLM=O and water, and (c) a direct attack on DNA giving Fe(IV)BLM=O and water.

Route (a) that gives rise to free $^{\bullet}$OH has been ruled out by the inefficiency of external $^{\bullet}$OH scavengers (Müller et al. 1972; Gutteridge and Shute 1981; Rodriguez and Hecht 1982; Dedon and Goldberg 1992), the absence of major amounts of damaged DNA bases (Gajewski et al. 1991) and the formation of base propenals (Burger et al. 1980, 1982a, b; Giloni et al. 1981; Hecht et al. 1990) which are not formed upon $^{\bullet}$OH-attack and subsequent peroxyl radical reactions in the absence of BLM (Rashid et al. 1999). Theoretical considerations favor pathway (c) (Neese et al. 2000; for quantum-mechanical calculations on Fe(III)OOH see also Ensing et al. 2003).

Any mechanism has to take into account that base propenals and unaltered bases are released (Giloni et al. 1981), but the former only in the presence of O_2 (Burger et al. 1982b). In the presence of O_2, base propenals, free bases and strand breaks are formed in about equal amounts (Burger et al. 1982a, b). Kinetically, strand breakage and the elimination of H2′ (Wu et al. 1983) precede base propenal release (Burger et al. 1986). Besides base propenals, DNA fragments with 3′-phosphoglycolate and 5′-phosphate termini are the products of this reaction (Sugiyama et al. 1985b; Giloni et al. 1981). Together with the liberation of a free base, an ALS is generated (Povirk et al. 1977) that has been attributed to the 4′-keto-1′-aldehyde lesion (Sugiyama et al. 1985a; Rabow et al. 1986, 1990; Wu et al. 1985b). Base release does not occur at random (Gua : Ade : Cyt : Thy = 1 : 1.7 : 6 : 12; Povirk et al. 1978). Frank SSBs and ALSs are formed in about equal amounts (Povirk et al. 1977). Moreover, DSBs are observed as a primary product [SSB/DSB = 9 (Povirk et al. 1977); SSB/DSB = 3.3-5.8 (Absalon et al. 1995); SSB/DSB = 6 and strongly modified by changes of the linker region (Boger et al. 1995)]. Thus, the two SSBs that give rise to a DSB must be formed at very close distance. BLM is recycled with a turnover of only 5-10 (Owa et al. 1990), that is, there is considerable self-destruction.

A mechanism that accounts for the major aspects of the BLM chemistry is shown below (for some potential variations see Rashid et al. 1999). Upon reacting 'activated BLM', Fe(III)BLMOOH, with DNA a *C*(4′) radical and a Fe(IV)BLM=O species are formed [reaction (50)]. The latter is still attached to DNA. In the absence of O_2 and/or in competition to an O_2-addition to the *C*(4′) radical [reaction (53)], the Fe(IV)BLM=O species may add to the *C*(4′) radical [reaction (51)]. One also may consider an outer-sphere one-electron oxidation by the Fe(IV) species which would be equivalent as the resulting products are concerned. The final

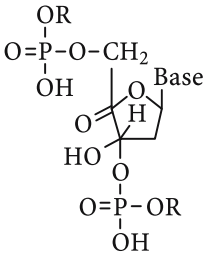
product are Fe(III)BLM and the 4′keto-1′aldehyde lesion mentioned above [reaction (52)]. For a further reaction, the Fe(III)BLM has to be reactivated.



The formation of base propenals is not as straight-forward. From the above, it is clear that O_2 is required for the formation of the base propenals. After the formation of the *C*(4′) peroxyl radical [reaction (53)], the Fe(IV)BLM species has to interact with this peroxyl radical, possibly by forming an adduct [reaction (54)]. The latter then decomposes and subsequently yields the products, the 3′-phosphoglycolate, the base propenal and the 5′-phosphate terminus [reactions (55)–(57)]. When reaction (53) is carried out with $^{18}O_2$, ^{18}O is incorporated into the 3′-phosphoglycolate (McGall et al. 1987), while the carbonyl oxygen of the

base propenal originates from water (McGall et al. 1992). In this reaction the 2′*R*-proton is eliminated when forming the C-C double bond of the base propenal (Ajmera et al. 1986). The sequence (56) followed by (57) accounts for the observation that stand breakage and H2′ release precedes the formation of base propenals.

The above scheme does not fully reflect the present view expressed in the literature, but retains essential elements. In the literature one usually finds as one of the intermediates the 4′-hydoperoxide. This is a species that should also be formed when the *C*(4′)-peroxyl radical reacts with $O_2^{\bullet -}$, conditions that prevail in the γ-radiolysis of DNA in oxygenated aqueous solution, but under these conditions, no trace of base propenals is formed (Sect. 12.4.4). In early mechanistic proposals, the geminal phosphatohydrine has been assumed to be an intermediate with the substantial lifetime of tens of minutes.



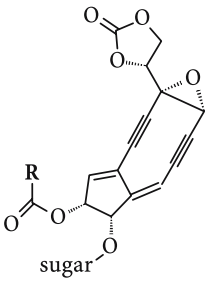
4'-Keto-3'-phosphatohydrine

Yet, the corresponding geminal chlorohydrines have a lifetime of less than a few μs (Mertens et al. 1994). The related $(\text{i-PrO})_2\text{P(O)OC(CH}_3)_2\text{OH}$ decays with $k \geq 3 \times 10^4\ \text{s}^{-1}$, and the data on the peroxyl radical chemistry of trimethylphosphate (Schuchmann, von Sonntag 1984) will have to be reinterpreted in so far as the decay of $(\text{CH}_3\text{O})_2\text{P(O)CH}_2\text{OH}$ into dimethylphosphate and formaldehyde must have occurred during the bimolecular decay of the peroxyl radicals, i.e. on the submillisecond time scale.

12.9.3 Enediyne Antibiotics

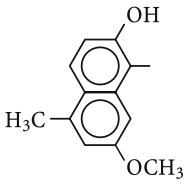
The DNA-binding antibiotics, neocarzinostatin (NCS), dynemicin, calicheamicin and esperamicin have all in common that they have an enediyne as a structural element, although they are otherwise very different (for a review see Nicolaou and Dai 1991). Their formulae are given in Fig. 12.13.

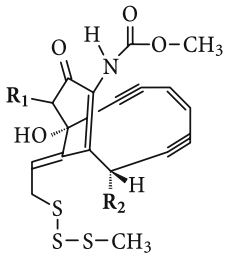
NCS intercalates with the aromatic moiety into the minor groove (Povirk et al. 1981; Dasgupta and Goldberg 1985; for the kinetics with the model poly(dA-dT) see Dasgupta et al. 1985) as do the other enediyne antibiotics, e.g., esperamicin (Yu et al. 1994). In the case of calicheamicin, the lifetime of the dihydrothiophene intermediate, formed in reaction (58), is sufficiently long to contribute to the sequence-selective binding and thus to the observed cleavage pattern (Chatterjee et al. 1995).



Neocarzinostatin

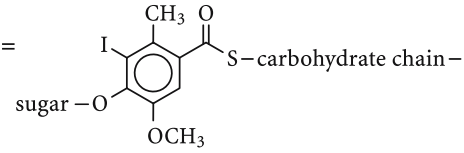
R =

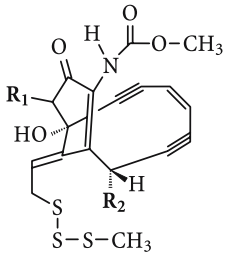




Calicheamicin γ_1

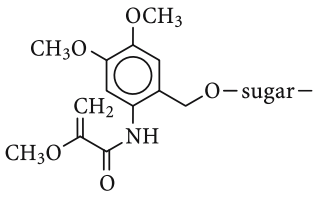
$R_1 = H$ $R_2 =$



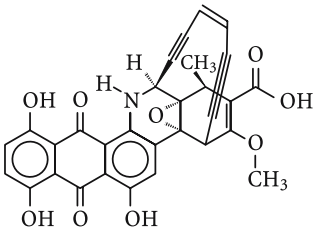


Esperamicin A_1

$R_1 =$

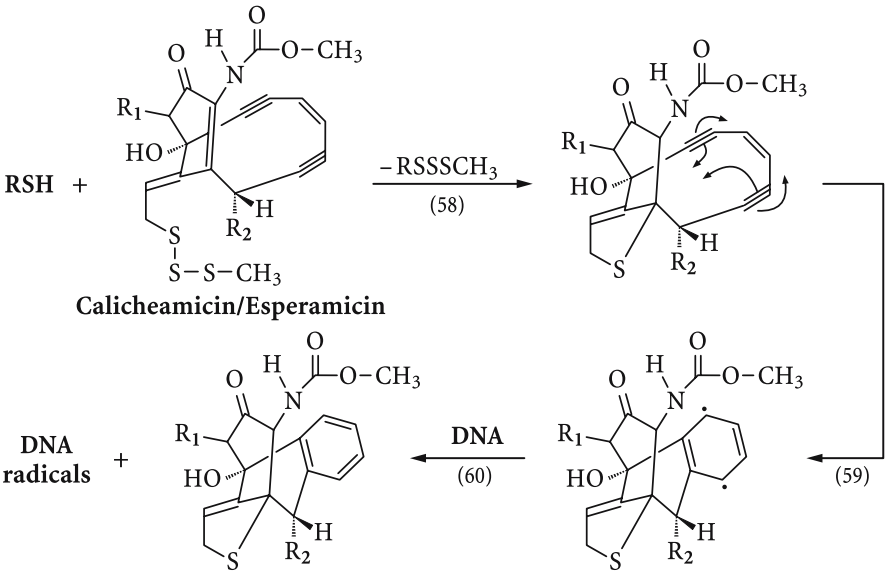


$R_2 =$ carbohydrate chain–

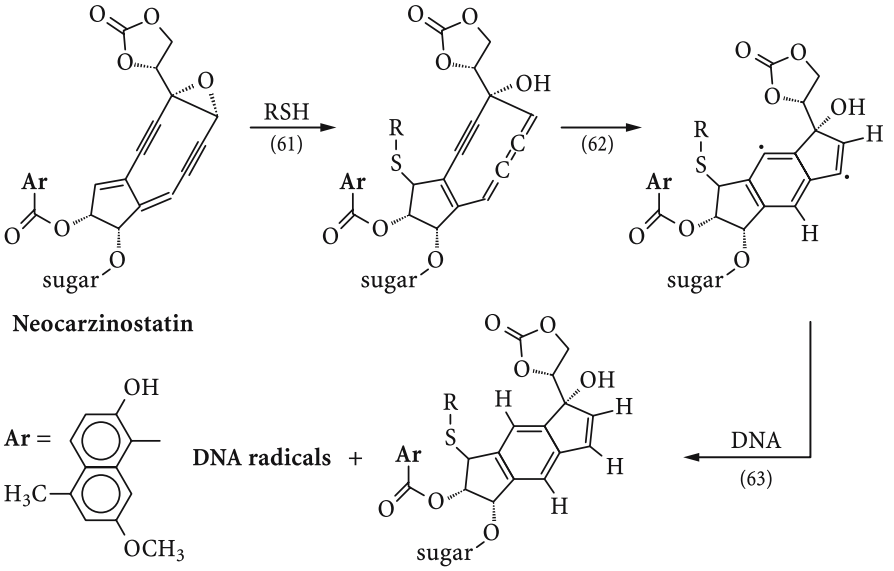


Dynemicin

Fig. 12.13. Structures of some of the enediyne antibiotics. For details of the carbohydrate groups, see Dedon and Goldberg (1992)



Triggered by a thiol, they undergo very amusing rearrangements leading to biradicals. Examples are shown in reactions (58) and (59) and reactions (61) and (62). For NCS and 2-mercaptoethanol, the trigger rate constant is 4 $dm^3\ mol^{-1}\ s^{-1}$ at pH 7.8 (Povirk and Goldberg 1983).



Upon the thiol-induced breakdown of the enediyne system, two vinyl-type radicals are formed. Although such radicals are good H-abstractors (Chap. 6.5), there is considerable selectivity in the sequence tertiary > secondary > primary

H-atoms (Mertens and von Sonntag 1994). In contrast to BLM, where H4′ is attacked, it is mainly the H5′ that is abstracted by NCS. This points to a highly stereoselective topochemical reaction, since the tertiary H1′ and H4′ would be the thermodynamically, and at equal accessibility, also the kinetically favored targets. This is supported by marked differences between esperamicin A and esperamicin C (Epstein et al. 1997). The former cleaves only at *C*(5′) while the latter also cleaves at *C*(4′).

With NCS and in the presence of O_2, a free 3′-phosphate and a DNA-bound Thy-5′-aldehyde is the major lesion (Kappen et al. 1982; Kappen and Goldberg 1983), and with $^{18}O_2$ the label is introduced in that position (Chin et al. 1984). With a 75% preference for T, the base selectivity is very high [T >> A >> C > G) (Takeshita et al. 1981; Goldberg 1991)]. Hydroxylation at *C*(4′) giving rise to the C4′-AP is the other pathway that complements the Thy-5′-aldehyde lesion (Saito et al. 1989). Nitroaromatic radiation sensitizers can replace O_2 (Kappen and Goldberg 1984; Goldberg 1987; Chin et al. 1987; Kappen et al. 1989), but the Thy-5′-aldehyde formation is largely suppressed and 3-formylphosphate (only 10-15% in the presence of O_2) is now an important product (for model systems, see Chap. 6.3, for the hydrolysis of a formyl phosphate see Schuchmann and von Sonntag 1984).

In the absence of O_2, there is little if any strand breakage; instead the drug binds covalently to DNA (Povirk and Goldberg 1982, 1984) and induces, under certain conditions, interstrand DNA cross-links (Xu et al. 1997) with the same base specificity as SSB formation in the presence of O_2 (Povirk and Goldberg 1985a).

When one of the two vinyl-type radicals has reacted with DNA, there still remains a second highly reactive radical for further reaction. If the rate constants with 2-PrOH (vinyl radical: $k = 2 \times 10^5$ dm^3 mol^{-1} s^{-1}; phenyl radical: 1.2×10^7 dm^3 mol^{-1} s^{-1}; Mertens and von Sonntag 1994) are a good guide O_2 stands only a small chance to compete with H-abstractions from the sugar moiety. An attack at both DNA strands leads to a clustered lesion. Mechanistically, NCS and BLM, where the phenomenon of a clustered lesion is also observed, differ in so far as in NCS the two radicals of the biradical set two close-by lesions, while in BLM the drug has to reactivated for the second action and potentially subsequent ones. NCS can only give rise to two damaged sites, and the drug cannot be recycled.

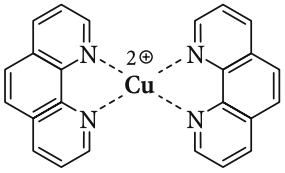
The second action of NCS that gives rise to a DSB or an ALS seems to be not as regiospecific as the first step that creates only a SSB. Attack at *C*(1′), *C*(4′) and *C*(5′) is envisaged. As discussed above, attack at the former two positions leads to the release on unaltered bases, and this is indeed observed (Hatayama and Goldberg 1980; Povirk and Goldberg 1984).

Interestingly, the nature of the thiol (GSH vs other thiols) that is used to trigger the reaction, influences to some extent the pathways that are subsequently taken (Povirk and Goldberg 1985b, 1986). NCS may also be activated by $CO_2^{\bullet-}$ (Favaudon et al. 1985). Here, three $CO_2^{\bullet-}$ are required for an activation, and the preferred site of attack is now H1′.

In the duplex d(AGCGAGC*G), NCS abstracts H1′ at the C* position, whereby Cyt is released and the sugar moiety transformed into the 2-dRL lesion (Kappen and Goldberg 1989).

12.9.4 Bis(1,10-phenanthroline)-Copper Complex

The bis(1,10-phenanthroline)-Cu(II) complex, when reduced to the Cu(I) complex [$(OP)_2Cu^+$], cleaves DNA upon the addition of H_2O_2 in oxygenated solution (Sigman et al. 1979; Que et al. 1980; Downey et al. 1980; Marshall et al. 1981a, b; Reich et al. 1981; Sigman 1986; Goyne and Sigman 1987; Meijler et al. 1997; for reviews that also report details of the interesting biochemistry see Sigman 1990; Sigman and Chen 1990; Sigman et al. 1993b, 1996).

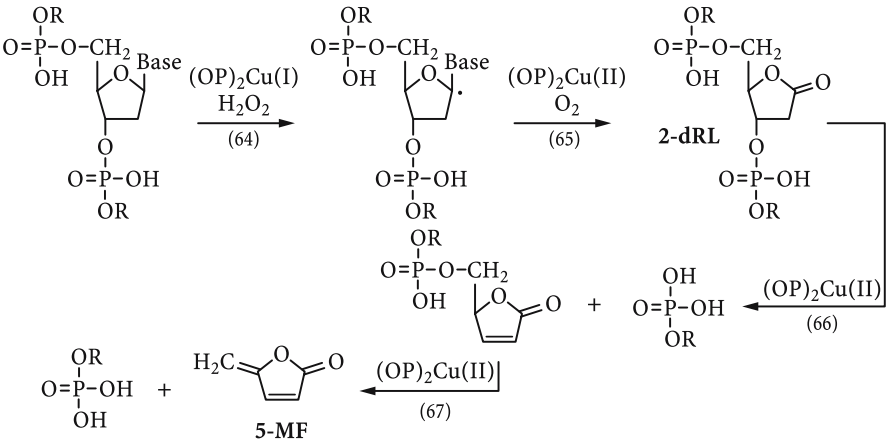


Bis(1,10)-phenanthroline-Cu(II)

The mono(1,10)phenanthroline–Cu(I) complex ($OPCu^+$) also cleaves DNA, but has to be tethered to DNA for showing nuclease activity, while $(OP)_2Cu^+$ can intercalate with one of the ligands (Sigman et al. 1996). Thus $(OP)_2Cu^+$ requires dsDNA, where it inserts into the minor groove (Marshall et al. 1981a; Oyoshi and Sugiyama 2000). On the other hand, $OPCu^+$ can be used with advantage for specifically directing the nuclease activity, and with the help of Fis-tethered $OPCu^+$ a new Fis binding site has been identified (Pan et al. 1996). Similarly, the *E. coli* Trp suppressor has been transformed into a site-specific nuclease (Sutton et al. 1993; Chen and Sigman 1987).

Typical $^{\bullet}OH$-scavengers suppress this reaction of $(OP)_2Cu^+$ (Que et al. 1980); yet, acetate and benzoate seem to be equally efficient, despite the fact that acetate is nearly two orders of magnitude less reactive towards $^{\bullet}OH$ than benzoate ($k = 7 \times 10^7$ dm^3 mol^{-1} s^{-1} vs $k = 5 \times 10^9$ dm^3 mol^{-1} s^{-1}; Buxton et al. 1988), and obviously it is not a freely diffusing $^{\bullet}OH$ that is responsible for the reaction. The reaction is also suppressed by Cu-complexing compounds and by transition metal ions such as Zn^{2+}, Co^{2+}, Cd^{2+} and Ni^{2+} that form stable complexes with 1,10-phenanthroline (Que et al. 1980) and also by competitive intercalators such as ethidium bromide and 2,9-dimethyl-1,10-phenanthroline (Reich et al. 1981). Interestingly, compared to its parent, the latter is inactive. NADH may serve as a reductant, but $O_2^{\bullet -}$ seems to be a salient intermediate in this cleavage reaction, because cleavage is fully suppressed in the presence of SOD (Reich et al. 1981).

In the reaction of $(OP)_2Cu^+/H_2O_2$ with dsDNA, 2-dRL and 5-MF are major products. Originally, they have been thought to have a common precursor, the *C*(1′) radical [reactions (64)-(67)].



This view has been challenged, and using a hexamer duplex as dsDNA model it has been shown that the 2-dRL is a perfectly stable lesion at pH 7, and at room temperature it only eliminates the β-phosphate group at pH 9.9 [cf. reaction (66); heating at 90 °C for 30 min afforded 5-MF; Oyoshi and Sugiyama 2000]. In this system, scission is due to radical precursors at *C*(4′) and *C*(5′).

Realizing that 2-dRL causes such a small dsDNA segments to melt, i.e., the oxidized hexamer duplex/$(OP)_2Cu$ complex could fall apart, before $(OP)_2Cu$ could exert its catalytic activity, experiments have been carried with a 33mer duplex with an inserted 2-dRL lesion and a $(OP)_2Cu^+$ conjugate that cleaves at the same position, and the cleavage kinetics were followed (Bales et al. 2002). The cleavage rate with the 2-dRL lesion already build-in was 5.6×10^{-4} s^{-1} and the oxidation of the intact duplex 1.9×10^{-5} s^{-1} indicating that the $(OP)_2Cu$-catalyzed β-elimination [reaction (66)] is faster than the oxidation [reactions (64) and (65)], and it has been concluded that the original mechanistic proposal by Sigman is supported by these experiments.

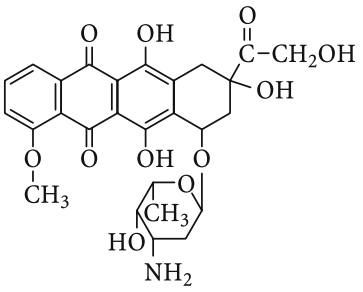
It is commonly assumed (see also Uesugi et al. 1982) that the intercalated $(OP)_2Cu$ complex upon reduction to Cu(I) by an added thiol and reaction with H_2O_2 gives rise to •OH that abstracts H1′ in a rather site-specific reaction. H1′ is quite hidden inside the minor groove, and •OH-reactions are typically not as site-specific. One is hence tempted to speculate whether or not the observed specificity is due to another species, such as Cu(I)OOH or Cu(III). For example, an Fe(III)OOH intermediate is made responsible for the H4′-abstraction in BLM (Sect. 12.9.2). The $(OP)_2Cu$ reaction is suppressed by acetate, a poor H-donor with a relatively low rate constant towards •OH. This remarkable suppression could be due to an interaction with Cu(III) by electrostatic binding, ET and subsequent decarboxylation (for some properties of aqua-Cu(III) see Chap. 3.4). Details are as yet not known.

12.9.5
Transition Metal Desferal Complexes

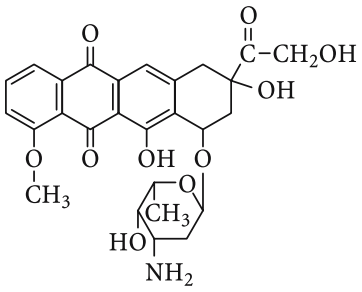
In the presence of H_2O_2 and a reductant such as 2-mercaptoethanol, the desferal complex of Cu(II), Co(III) and Ni(II) cleave plasmid DNA, but the corresponding Fe(II) complex is inactive (Joshi and Ganesh 1992; for the formula of desferal see Chap. 2.5). This cleavage is inhibited by •OH-scavengers such as mannitol. With the help of synthetic ODNs it has been shown that the Cu(II) desferal complex has only a marginal sequence preference for the cleavage reaction (CG>AT), while the Ni and Co complexes cleave DNA only at CG sites (Joshi and Ganesh 2004). This clearly indicates that some binding to DNA must occur, and some suggestions are made in the cited papers (for further studies see Joshi and Ganesh 1994a,b; Joshi et al. 1994).

12.9.6
Adriamycin

Adriamycin and 11-deoxyadriamycin when complexed to DNA cleave DNA in the presence of Fe^{3+} (Muindi et al. 1984).



Adriamycin



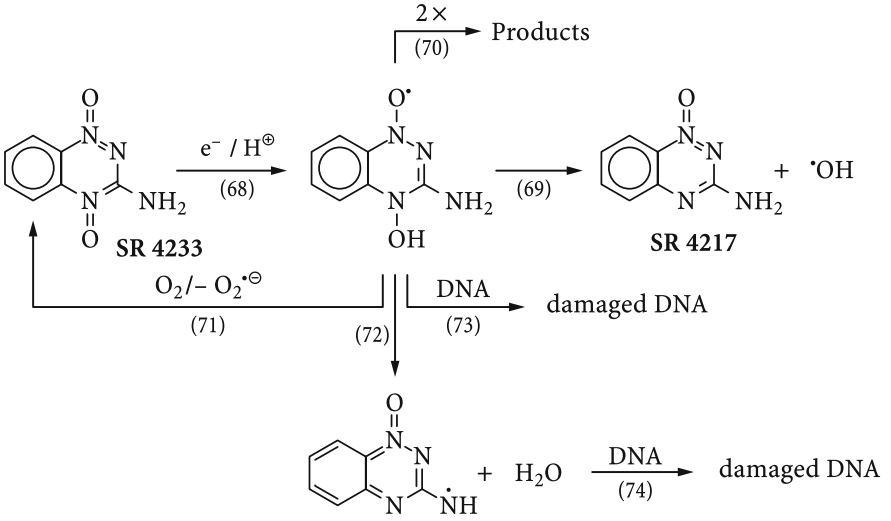
11-Deoxyadriamycin

Adriamycin is considerably more reactive than its 11-deoxy derivative. The reactive intermediate in this DNA cleavage reaction is thought to be the •OH radical. In order for a Fenton reaction to proceed, Fe^{3+} would have to be reduced to Fe^{2+}. No typical reductants are required for this reaction to proceed, and thus adriamycin would have to provide the reduction equivalent, e.g., through its hydroquinone moiety. This would be in agreement with the much lower efficiency of the 11-deoxy derivative which lacks a strongly reducing function. The intermediacy of •OH has been concluded from a spin-trapping experiment with DMPO. This test is, however, not as straightforward as commonly believed, because other strong oxidants can give rise to the •OH-adduct via the DMPO radical cation (von Sonntag et al. 2004b). Thus, it is well possible that the DNA/adriamycin/Fe^{3+} complex reacts with H_2O_2 to give an Fe(III)-hydroperoxide. Such an intermediate is made responsible for the site-specific reaction of BLM (Sect. 12.9.2).

12.9.7 Tirapazamine

Tirapazamine (SR 4233) is the lead compound to a class of bioreductive anticancer drugs that makes use of the fact that solid tumors are hypoxic (Brown 1990, 1993; Brown and Wang 1998) and clinical phase III trials are under the way (Gandara et al. 2002). With an anticancer drug that is that far advanced in clinical trials one would like to know the mechanism of its action. Obviously, this has attracted many research teams that are expert in different techniques to tackle the problem. It will be shown below that there is now a host of first-class information, but it is as yet difficult, if not impossible, to arrive at a conclusive mechanism of its action.

Neither the drug itself nor its two-electron reduction product SR 4317 are toxic as long as the cell is oxygenated [reactions (68) and (71)].



Under hypoxic conditions, cellular enzymes reduce the benzotriazine di-*N*-oxide [(reaction (68); P450 reductase; Cahill and White 1990 and NADPH may be involved; Walton et al. 1992; Wang et al. 1993]. Upon microsomal reduction of tirapazamine the radical formed in reaction (68) has been identified by EPR (Lloyd et al. 1991). Using the pulse radiolysis technique, it has been shown that this radical has a pK_a of 6 (Laderoute et al. 1988), and it is the protonated form that undergoes the DNA damaging reaction (Wardman et al. 2003). The rate constants of the bimolecular decay of the radical [reaction (70)] has been found to be 2.7×10^7 dm^3 mol^{-1} s^{-1}. The reaction with its anion is somewhat faster (8.0×10^8 dm^3 mol^{-1} s^{-1}), while the deprotonated radicals do not react with one another at an appreciable rate. From another set of pulse radiolysis data, a first-order process has been extracted ($k = 112$ s^{-1}) that has been attributed to the water elimination reaction (72), and the tirapazamine action on DNA [reaction (74)] has been considered to be due to the resulting radical (Anderson et al. 2003).

The alternative, the elimination of •OH [reaction (69)] has been considered to be much less likely. As has been discussed in Chapter 6.9, water elimination reactions may considerably change the properties of a radical. This also applies to the tirapazamine system. The primary 'reducing' tirapazamine radical turns into an oxidizing one upon water elimination. Its rate constant with dGMP has been determined at 1.4×10^8 dm^3 mol^{-1} s^{-1}. It has been reported to react also with 2-deoxyribose by H-abstraction at 3.7×10^6 dm^3 mol^{-1} s^{-1} (see also Shinde et al. 2004). At first sight, this is very surprising for a radical for which many mesomeric forms can be written. However according to DFT calculations, H-abstraction from the sugar moiety is exoenergetic and H-transfer would occur to the exocyclic NH function, the site of highest spin density (Naumov and von Sonntag 2005, unpublished results).

It is commonly agreed that the one-electron reduced drug is capable of inducing DNA strand breaks, even DSBs (Brown 1993). With DNA in aqueous solution, SSBs dominate considerably over DSBs (Jones and Weinfeld 1996). The major detectable lesion (32% of the 3′ ends) has been identified as the phosphoglycolate. Its yield dramatically increases with increasing tirapazamine concentration, and it has been suggested that this reaction is caused by an abstraction of H4′ by the activated drug and donation of an oxygen atom (possibly the *N*-oxid oxygen) to the *C*(4′) radical by a second tirapazamine. This reaction would be reminiscent of the action of nitroaromatic sensitizers (Chap. 6.3), but quite different from a mere one-electron oxidation of a reducing radical by tirapazamine. For example, when the *C*(1′) radical is generated photolytically in an adequately substituted ss/dsODNs, it is rapidly oxidized by the drug to the 2-dRL lesion (Daniels et al. 1998; Hwang et al. 1999b), thereby another drug molecule is activated (Wardman et al. 2003). The rate of reaction with the ssDNA model is 2.5×10^8 dm^3 mol^{-1} s^{-1}, and the rate of reaction drops to 4.6×10^6 dm^3 mol^{-1} s^{-1} with the dsDNA model (Hwang et al. 1999b). This dramatic drop in rate may be due to the fact that the *C*(1′) radical is deeply hidden in the minor groove (Sect. 12.2), and thus difficult to approach.

SSB formation is suppressed by typical •OH-scavengers such as MeOH, DMSO and *t*BuOH at high concentrations (0.1 mol dm^{-3}); but the suppression is only partial (Daniels and Gates 1996). The involvement of (free) •OH, as suggested, cannot be the major cause of the DNA damage, since in the reaction with DMSO (in the absence of DNA) the effectiveness of xanthine/xanthine oxidase activated tirapazamine in forming methanesulfinic acid is only in the 1% range (based on added tirapazamine; 92% is the theoretical value if full conversion is achieved; Chap. 3.2; for a caveat that the xanthine/xanthine oxidase system as such may nick DNA under certain conditions via •OH see Jones and Weinfeld 1996). The formation of some free •OH is supported by spin-trapping experiments (Patterson and Taiwo 2000), but strongly oxidizing species other than •OH give also rise to the this spin-adduct (Sect. 12.9.5). Tirapazamine is much more effective in creating base damage (Birincioglu et al. 2003) then can be accounted for by free •OH that reacts with DNA (for the competition kinetics as a function of the scavenger capacity of the solution see Udovicic et al. 1991a).

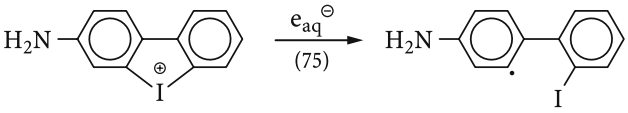
Little if any base damage was detected using the ^{32}P-postlabeling assay (Jones and Weinfeld 1996). Yet, this assay only records a limited number of such lesions

(Tg, for example; Weinfeld and Soderlind 1991). The pattern of DNA damage (bases and sugar moiety) as recognized by repair enzymes is very similar to that given by free •OH (produced by ionizing radiation; Kotandeniya et al. 2002), but the *R*/*S* ratios of cG and even more so for cA deviate markedly from that of •OH-induced reactions (Birincioglu et al. 2003). In addition, the product ratios of typical pairs such as 5OH5,6H_2T/Tg (tirapazamine: 15.7 vs •OH: 0.75; data from N_2O-saturated dsDNA solutions in Table 12.5) or FAPY-G/FAPY-A (4.1 vs 1.4) differ markedly.

The inefficiency of even high •OH scavenger concentrations to fully suppress the reaction (Daniels and Gates 1996) could be accounted for if the activated drug would intercalate into DNA and in this bound state would release •OH. However, there is no indication that tirapazamine intercalates into DNA (Poole et al. 2002); targeting tirapazamine to DNA via an acridine linker increases DNA strand breakage considerably in vitro (Anderson et al. 2003a) and in vivo (Delahoussaye et al. 2003). The activity of the drug in cellular systems strongly argues against free •OH as the main cause of its DNA-damaging effects (Chap. 3.3), and only if tirapazamine were activated in the very neighborhood of DNA and would release •OH instantaneously some of the •OH could stand a chance of reacting with DNA. It is recalled that the diffusion length at high scavenger concentrations such as prevail in a cell is only a few Å. There are not only problems with the •OH hypothesis, but also a freely diffusing oxidizing tirapazamine radical poses problems. Its 50-fold faster rate with G as compared to 2-deoxyribose would lead one to expect a preference in G oxidation. Moreover, its redox potential of +1.31 V (Anderson et al. 2003b) is not sufficient to oxidize any other nucleobase and the formation of the various products derived from A, T and C (Birincioglu et al. 2003) cannot be explained on the basis of one-electron oxidation reactions. Certainly, more work is required to elucidate the primary processes of this most interesting DNA damaging drug.

12.9.8 Diphenyleneiodonium Cations

o,*o*′-Diphenyleneiodonium ions intercalate into DNA, and upon reduction with e_{aq}^- a highly reactive phenyl-type radical (Chap. 6) is formed [reaction (75)] which undergoes H-abstraction from the sugar moiety, preferably from *C*(1′) (Razskazovskiy 2003).



12.10 Charge and Excitation Transfer Through DNA

There is a long-standing interest in the question of charge and excitation transfer through DNA. This largely resulted from an attempt to protect or to sensitize DNA against the effects of ionizing radiation in radiotherapy. The concept of

radiation sensitizers has originally been based on the assumption that relatively low concentrations of a sensitizer, typically an electron-affinic compounds (for reviews see Wardman 1977, 1984, 1987), may scavenge the electron (migrating through DNA?; Adams and Dewey 1963) or even the dry electron (Bakale and Gregg 1978) and thus prevent electron-hole recombination that was thought not to lead to DNA damage. The fact that relatively low concentrations of electron-affinic radiosensitizers are required to cause a biological effect led to the concept of a fast and over larger distances travelling presolvated electron. The observation that the presence of DNA strongly modified charge recombination in pulse-irradiated ice (Warman et al. 1980) and the conductance data obtained with pulse-irradiated DNA (van Lith et al. 1983, 1986a, 1986b; Warman et al. 1996) gave new weight to this concept. In the pulse radiolysis of solid DNA, the kinetics of the intermediates strongly depend on the hydration of the DNA samples (O'Neill et al. 1989). The luminescence from pulse-irradiated "dry" and hydrated DNA noticeably depends on the composition of the DNA and most of it occurs during the pulse (Al-Kazwini et al. 1988), somewhat at variance with earlier reports (Adams and Jameson 1980; Lillicrap and Fielden 1969). The in-pulse luminescence decreases with increasing addition of misonidazole and drops to half of its value at one misonidazole molecule per base pair (O'Neill 1989), and it is concluded that radiation-induced energy migration may occur within DNA. This led to an estimate of an electron migration distance of approximately 20 base pairs (Al-Kazwini et al. 1991). A study using three different electron acceptors whose reduction potential varies from −0.5 to −0.2 V shows a strong influence of the quenching efficiency with the reduction potential (Al-Kazwini et al. 1994), and it is concluded that electron migration distances of even at least 300 base pairs are likely (biological radiosensitization shows a similar dependence on the reduction potential, cf. Adams et al. 1979; Naylor et al. 1991). Yet in aqueous solutions, ET from DNA radical anions to intercalated electron-affinic radiosensitizers proceed only over a distance of about three base pairs (Anderson et al. 1991). Short-range ET (2–8 bp) has also been concluded from the radical yields of γ-irradiated ODNs at 4 K (Debije et al. 1999). The observation that in DNA γ-irradiated at low temperature the hole ends up at G (Wang et al. 1994; Sevilla et al. 1991; see also below) is clear evidence that charge (here: hole) migration must occur over a certain distance. Thus, a host of mobile entities including excited states (e.g., Smith 1979) have been considered to explain the observed phenomena. Three important questions could not be answered by most of these early studies: (1) how fast is the electron (hole) transfer, (2) over which distance does it occur and (3) what is the mechanism of hole/electron transfer.

In recent years, the interest in hole and electron transfer has so dramatically increased that it is no longer possible to report details adequately in the limited space available here. Therefore, attention is drawn to some reviews (Grinstaff 1999; Symons 1997; Giese 2000; Schuster 2000; O'Neill and Barton 2004; Kawai and Majima 2004; Schuster and Landman 2004; Nakatani and Saito 2004; Douki et al. 2004).

Long-range charge transfer by tunneling can be described by Eq. (76), where k is the rate of charge transfer, β the attenuation coefficient that qualifies the

damping effect of the interspersed medium, and R_{AD} the distance between donor and acceptor.

$$k \propto e^{-\beta R_{DA}} \tag{76}$$

When the charge transfer occurs high β value ranging between 1.0 and 1.5 $Å^{-1}$ are observed (Beratan et al. 1997; Priyadarshy et al. 1996). This is, for example, found for charge transfer through proteins (Nocek et al. 1996). Such high β values have also been reported for DNA under certain conditions, e.g., (Lewis et al. 1999a, 1999b, 2000a; Brun and Harriman 1994; Meade and Kayyem 1995; Cai and Sevilla 2000; Messer et al. 2000; Fukui and Tanaka 1997; Krider and Meade 1998; Meggers et al. 1998a, b).

Lower β values around 0.6 ± 0.1 were observed for the charge transfer from excited 2-aminopurine to G through interlacing adenines (Wan et al. 2000) and in DNA hairpins (Lewis et al. 1997; Lewis and Letsinger 1998; for a review see Lewis et al. 2001).

However, very low β values (0.2 $Å^{-1}$ and even lower) have also been reported (Murphy et al. 1993; Arkin et al. 1996; Kelley et al. 1997; Holmlin et al. 1997, 1998; Henderson et al. 1999; Ly et al. 1999; Kelley and Barton 1999; Kelley et al. 1999; Giese et al. 1999; Schiemann et al. 2000; Stemp et al. 2000; for studies on ET through solid DNA see, e.g., Eley and Spivey 1962; Okahata et al. 1998; Fink and Schönenberger 1999; Porath et al. 2001).

A small β value of ≈ 0.1 $Å^{-1}$ indicates that the ET rate only weakly depends on the distance between donor and acceptor. It has been pointed out (Grozema et al. 2000) that two different electron transport mechanism may give rise to such a weak distance dependence, the “molecular wire” mechanism (Turro and Barton 1998; Mujica et al. 1999), and the “incoherent hopping” mechanism (Jortner et al. 1998; Bixon et al. 1999). In the “molecular wire” mechanism, donor and acceptor are strongly coupled to one another through the intervening bridge, and the charge can travel through this π-way. In the "incoherent hopping" mechanism the charge travels in a multistep process "hopping" between GC pairs that act as localization sites until it reaches the acceptor. The rate of this process does not decay exponentially with the distance and hence β is not a suitable parameter. Here, the logarithm of the rate of charge transfer is proportional to the number N of hopping steps; Eq. (77).

$$\ln k \propto -\eta \ln N \tag{77}$$

The power parameter η equals 2 for unbiased diffusive hopping from the donor to the acceptor, and between 1 and 2 for a direction-biased random walk process (Priyadarshy et al. 1996).

Quantum-mechanical calculations (Grozema et al. 1999, 2000; Berlin et al. 2000) that take the molecular wire and quantum mechanical channels (cf. Fig. 12.14) into account are capable of explaining the wide range of β values reported. This theory also allows for the observed drastic dependence on the rate of hole transfer on the number and nature of the nucleotide pairs AT vs GC between the donor ($G^{\bullet+}$) and the acceptor (GGG) as reported by Meggers et al.

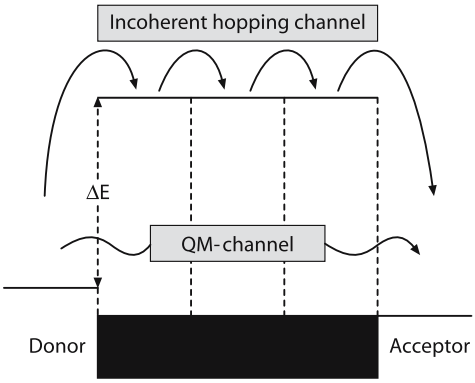


Fig. 12.14. Schematic representation of charge transfer in a donor-DNA-acceptor system via a quantum-mechanical (QM) channel and via incoherent hopping according to Grozema et al. (1999, with permission)

(1998b). In this system, the β value is 0.7 when AT pairs are between donor and acceptor but the ET rate increases by two orders of magnitude, when a CG pair is interlaced instead of an AT pair. The model by Berlin and Siebbeles takes into account that the ionization potential of a GC base pair is 0.55 eV lower than a AT bridge (and 0.75 eV lower than that of guanine; Hutter and Clark 1996). Neighboring GC pairs have even lower ionization potentials, and that of a GC triade has been calculated to be lower by as much as 0.7 V than a single GC pair (Saito et al. 1998). The energy gap of 0.55 eV between a GC and an AT pair determines the value of the injection barrier (ΔE in Fig. 12.14) which controls the injection of holes into the bridge in the experiment by Meggers et al. (1998b). In the other experimental studies mentioned above, a wide variety of hole donors which differ from a GC pair have been used. It has been shown that the fall-off parameter β decreases as the injection barrier decreases and reaches a limiting value of $\beta = 0.09\ Å^{-1}$ for $\Delta E = 0$ eV.

Further refinements of the model that take the potential trapping of the charge due to deprotonation (cf. Steenken 1997); for DFT calculations on proton transfer reactions see Li et al. (2001), for DFT calculations on base pairing abilities see Reynisson and Steenken (2002a, b) or other irreversible reactions are envisaged (Grozema et al. 2000). There is a continuing improvement of the hopping models (Giese and Spichty 2000; Schlag et al. 2000; Bixon and Jortner 2001, 2002; Olofsson and Larsson 2001; for the polaron drift model see Conwell and Rakhmanova 2000). The effects of dynamic motions of the base pairs within the molecular stack and sequence-dependent inhomogeneities in energetics and base-base couplings have also been explored (Treadway et al. 2002; Berlin et al. 2000, b; Ye et al. 1999). Twisting and other kinds of disorder reduce the transport rate significantly (Grozema et al. 2002; Berlin et al. 2001a). Most studies are concerned with DNA in its B-form, but hole transfer in also observed in Z-form DNA (Abdou et al. 2001). The hole can also move from duplex DNA to single-stranded DNA overhangs (Kan and Schuster 1999b).

Table 12.14. Effective energy of the positive charge (in eV) localized at the middle *G* in 5′-XGY-3′ (X, Y = G, A, C and T) sequences. (Senthilkumar et al. 2003)

Y	G	A	C	T
GGY	7.890	8.040	8.310	8.290
AGY	7.900	8.060	8.341	8.320
CGY	7.957	8.115	8.383	8.360
TGY	7.965	8.124	8.407	8.381

Among the nucleobases, G is most readily oxidized (in DNA: + 1.39 V at pH 7; Milligan et al. 2001a; the corresponding value for Guo has been determined at +1.29 V; Chap. 10.2). It has been noted (Milligan et al. 2001a) that this difference is inconsistent with the observation that G groups in polynucleotides are more easily oxidized than monomeric Gua (Seidel et al. 1996), but this discrepancy is not yet resolved. As mentioned above, GG and GGG sites have even lower redox potentials. Thus, hot spots in long-range one-electron oxidation of DNA are GG and GGG sites. There is a marked preference for the 5′-G contained in remote GG and the 5′-and central G in GGG sites (Hall et al. 1996; Stemp et al. 1997; Gasper and Schuster 1997; Saito et al. 1998; Nunez et al. 1999; for calculations. see Conwell and Basko 2001; Sugiyama and Saito 1996; Senthilkumar et al. 2003; Saito et al. 1998). The effective energy of the positive charge localized at the middle G calculated for in 5′-XGY-3′ (X, Y = G, A, C and T) sequences is given in Table 12.14.

It is seen from this table that a GGG site has the lowest energy, and in GG sites the base at the 3′-position influences the energy much more than the one at the 5′-position. This effect also an important influence on which G is oxidized in a GG site. With a pyrimidine at the 3′-position, it is largely the 5′-G that is oxidized. These calculations are met by experimental data (Hall et al. 1996; Ito et al. 1993; O'Neill et al. 2001; Melvin et al. 1995b; Sanii and Schuster 2000; Nakatani et al. 1999; Ito and Kawanishi 1997). At GGG sites, it is largely the G in the middle that is oxidized. An oxidation of the 5′-G is facilitated by a 5′-A (Senthilkumar et al. 2003). Again, this conclusion is met by experiments (Hall et al. 1996; Ito et al. 1993; Ito and Kawanishi 1997; Yoshioka et al. 1999). The forward rate from $G^{\bullet+}$ to a GG site through one interlaced A has been determined at ~5×10^7 s^{-1} and the reverse rate at 5×10^6 s^{-1} (Lewis et al. 2000b).

Base pairing (hydrogen bonding) of G with C reduces the reduction potential of G with respect of unpaired G (Kawai et al. 2000b). Substituting C by 5-MeC in DNA may reduce the oxidation potential of the opposing G as compared to the normal GC pair, and one might expect that such a site is capable of trapping the hole more effectively, but no effect was detectable (Kanvah and Schuster 2004).

Table 12.15. Ratio of damaged sites at GGG (P_{GGG}) and the G next to the strand break (P_G) as a function of the number of AT pairs between G and GGG. (Giese et al. 2001b)

Number of interlaced AT pairs	P_{GGG}/P_G
1	>250
2	30 ± 5
3	4.0 ±0.5
4	3.5 ± 0.5
5	3.0 ± 0.3
7	2.5 ±0.5

Detailed studies by Giese and his group clearly show the competition between the reaction of $G^{\bullet+}$ (generated by a neighboring strand break at *C*(4′)) with water and the migration of the hole along the DNA chain towards a GGG site (Giese et al. 2001a, b). As long as the next G is close, transfer to GGG is fast and occurs mainly by tunneling. With more than two interlaced AT pairs, the mechanism changes to a hopping mechanism. The ratio between the damaged primary G and that of the GGG site then becomes nearly independent of the number of interlaced AT pairs (Table 12.15).

As discussed above, the 5′-G is the preferred cleavage site in a GG trap, and in the GGG trap it is either the 5′-G or the middle G depending on the nucleobases neighboring the trap (for references see Davis et al. 2000; for quantum-mechanical calculations see Voityuk et al. 2000). Hole transfer always proceeds to the closest G, and only subsequently relaxation of the hole occurs which leads to the localization of the hole within the G tract (Davis et al. 2000). In triple-stranded DNA, the G of the third strand of the CG*G serves as an effective hole trap (Dohno et al. 2002; see also Kan and Schuster 1999a).

Upon photoexcitation of anthraquinone-linked duplex DNA, a hole is injected into the DNA. This has allowed the measurement of the traveling distance of the hole to a given GG in a large variety of dsODNs (Liu and Schuster 2003). With photosensitizers that are capable of oxidizing only G, a close distance between G and the sensitizer is required for efficient DNA damage, but for anthraquinone, which can oxidize also A, the A-hopping mechanism allows a hole transfer over a longer distance (Kawai et al. 2003b).

In a study on the 193-nm photoionization of a series of dsODNs, it has been shown that the charge transfer to G/GG sites occurs predominantly via intra- and not interstrand charge migration (O'Neill et al. 2001). This observation could be simulated by quantum-mechanical calculations.

For time-resolved studies, the hole can be injected in a laser flash experiment by a tethered (excited) electron acceptor and a sink (an electron donor) at a given distance (e.g., pyrene/phenothiazine; Ru-complexes/methylindole). Many of the

above-mentioned studies used this approach. These techniques are increasingly used to follow the rate of hole transfer under well-defined conditions (e.g., Takada et al. 2003a; Yoo et al. 2003; Kawai et al. 2003a).

The reduction potential of 8-oxo-G is only $E^7 = 0.74$ V/NHE (Steenken et al. 2000) and thus considerably lower than that of G. Consequently, 8-oxo-G acts as an efficient sink (Doddridge et al. 1998). ET to the radical cation of the sugar backbone is four times faster from 8-oxo-G than from G (Meggers et al. 2000). Distant 8-oxo-G may also serve a sink of the hole generated in DNA by the 2-aminopurine radical cation (Shafirovich et al. 2001) or pyrene radical cation (Kawai et al. 2001). Trapping of 8-oxo-$G^{\bullet+}$ by water has been suggested to be faster than 10^5 s^{-1} (Kawai et al. 2002). At 77 K, the rate of hole transfer from $G^{\bullet+}$ to the 8-oxo-G lesion is ~7 bp min^{-1}, and at room temperature this hole transfer leads to a steady-state of one 8-oxo-G lesion per 127± 6 bp, when $G^{\bullet+}$ is generated in aqueous solution by $Br_2^{\bullet-}$ (Cai and Sevilla 2003; for the EPR spectrum of 8-oxo-$G^{\bullet+}$ see Shukla et al. 2004; for the products formed upon oxidation of 8-oxo-G, see Chap. 10.13). The effects of neighboring Gs on the oxidation of 8-oxo-G seem to be similar as the oxidation of G in GG pairs (Prat et al. 1998).

For kinetic studies of hole transport, artificial sinks were developed. For example, N^2-cyclopropyldeoxyguanosine efficiently stops hole transport, although its reduction potential is not much lower (~0.13 V) than that of G/C (Nakatani et al. 2001). Rapid and irreversible cyclopropane ring opening has been suggested to terminate the hole transport. Similarly, N^6-cyclopropyldeoxyadenosine interrupts hole transport when placed between two G sites (Dohno et al. 2003). In contrast, N^2-phenyldeoxyguanosine suppresses the decomposition of the hole in its neighborhood (Nakatani et al. 2002a).

Hole transport through genomic DNA has been addressed, and it has been observed that it is suppressed by BamHI binding (Nakatani et al. 2002b). Mechanistically, this is due to hydrogen bonding of the positively charged guanidine moiety of BamHI to Gs in its DNA recognition sequence.

The charge transfer through DNA has been shown to play a role in the case of oxidative thymine cyclobutane dimer repair by a photoexcited tethered rhodium intercalator (Dandliker et al. 1997, 1998), in the photoionization of DNA by 193-nm photons (Melvin et al. 1995a, 1995b, 1998), the oxidation of the modified base 7-deazaguanine by photoexcited intercalated ethidium bromide (Kelley and Barton 1998). This process slows down considerably when more than two Ts intervene, and this has been suggested to be due to a proton-coupled ET (PT–ET) to another species, the neutral radical formed by deprotonation of the 2-aminopurine radical cation (for a theoretical treatment of PT, ET and PT–ET involving base radical cations, see Llano and Eriksson 2004). Femtosecond spectroscopy has been used to study charge transfer from photoexcited 2-aminopurine to DNA that occurs on the picosecond time scale varying strongly with the neighboring base (10 ps for G and 512 ps for hypoxanthin; Wan et al. 2000).

Using the $SO_4^{\bullet-}$ radical to form $G^{\bullet+}$ with the help of the pulse radiolysis technique and tethered pyrene as hole acceptor, it has been shown that the apparent transfer rates follow the order $G^{\bullet+}TG < G^{\bullet+}AC < G^{\bullet+}AG$ (Takada et al. 2003b). This means that A is a better medium for hole transfer than T.

Compared to hole transfer, ET through DNA has found less attention (Wagenknecht 2003). DFT calculations indicate, that the electron affinities of the nucleobases are U ≈ T > C ≈ I (hypoxanthine) > A > G with G being nearly 1 eV less electron affinic than U (Li et al. 2002). While in hole transfer, the most preferable trapping site is a GGG triplet, ET moves towards pyrimidine triplets whereby there is little energetic difference between XCY or XTY (X, Y = T or C) sites (Voityuk et al. 2001). Using brominated nucleobases (e.g., 5-bromo-6-hydroxy-5,6-dihydrothymine) as intramolecular electron scavenger indicates that excess electrons may travel through DNA about 11 bp at 77 K (Razskazovskii et al. 1997). Intercalated mitoxantron has been used to follow the transfer of excess electrons at 77 K through hydrated solid DNA (21 waters per nucleotide) and in frozen DNA solutions (Cai et al. 2000; Cai and Sevilla 2000), and similar β values (10 ± 1 bp min^{-1}) and ET distances were found for both systems (for further studies see Cai et al. 2001). From a series of similar experiments, a tunneling constant β of 0.8-1.2 $Å^{-1}$ has been derived at (Messer et al. 2000; Cai et al. 2002). It is concluded that at 77 K DNA is not an especially effective conduit for the transfer of excess electrons. The rate of ET through DNA to electron-affinic intercalators in aqueous solution depends on the free-energy change and distance (Anderson and Wright 1999). Migration of excess electrons does not proceed through ssDNA (Shafirovich et al. 1997).

The light-induced repair of cylobutane-type dimers by the enzyme photolyase is of major biological importance. This proceeds by ET from a flavin, and using a model system it has been shown that the electron is likely to be funneled through the DNA base stack (Schwögler et al. 2000).

12.11 Protection of DNA Against Free-Radical Attack

12.11.1 General Remarks

The trivial case, where DNA and a given additive compete for a reactive free radical, will not be discussed here, as long as competition kinetics are involved (a case in point would be the scavenging of DNA-damaging radicals by cell-permeable stable radicals such as nitroxides; Offer and Samuni 2002; Sasaki et al. 1998; Damiani et al. 2000). Due to polymeric properties of DNA, the competition kinetics are non-homogeneous (for a model that describes this situation adequately see Mark et al. 1989; Udovicic et al. 1991a; for an earlier spherical model see van Rijn et al. 1985), and competition with simple low-molecular-weight compounds follows indeed this model over a wide concentration range (Udovicic et al. 1991b, 1994). Any protection beyond this simple competitive radical scavenging can be assessed by calculating the contribution of competitive radical scavenging on the basis of the above model and the rate constants of a given radical with DNA and the competitor (k(•OH + DNA) = 4.5×10^8 dm^3 mol^{-1} s^{-1} - Liphard et al. 1990; Udovicic et al. 1994; 4×10^8 dm^3 mol^{-1} s^{-1} - Michaels and Hunt 1973; for compilation of •OH rate constants see Buxton et al. 1988). If the scavenger is

Table 12.16. Net charge (*Z*) dependence for the β values of the reduction in base release by some thiols and disulfides. (Zheng et al. 1988)

Compound	*Z* at pH 7	β value/10^{-3} mol dm^{-3}		
		Gua	Thy	Ade
2-Mercaptosuccinate	–2	1.2	1.9	2.5
2-Mercaptoethanesulfonic acid	–1		1.9	1.9
Glutathione (GSH)	–1	1.2	3.0	1.8
2-Mercaptoethanol	0	1.8	4.3	4.1
1,4-Dithiothreitol	0	1.9	4.5	–
Cysteamine	+1	4.0	7.0	8.0
WR 1065	+2	7.3	19	18
GSSG	–2	0.4	0.9	0.8
Bis-2-hydroxyethyldisulfide	0	1.3	2.4	2.5
Cystamine	+2	2.6	4.6	4.4

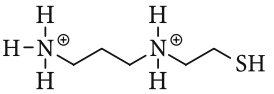
tightly bound to DNA, e.g., the bisbenzimidazole derivative *Hoechst 33258*, the high rate constant of the competitor may not be used for calculating the competition between DNA and the DNA-bound scavenger, because the tightly-bound scavenger is now also subjected to the same non-homogeneous kinetics as DNA (Adhikary et al. 1997a).

12.11.2 Thiols

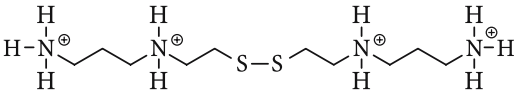
With thiols, protection against free-radical attack and repair of DNA damage (Sect. 12.12) is not always easy to disentangle in in vitro experiments and even more so in cellular systems (e.g., Murray et al. 1990). This has to be kept in mind, when some aspects of thiol protections are discussed here.

As in the case of poly(U), which has been studied as a model system in some detail (Chap. 11.2), there is a relationship of the rate of protection of DNA by thiols and disulfides and the charge of the sulfur compound due to ion condensation (for evidence of this phenomenon, see Smoluk et al. 1988b). As has been measured by following the protection of DNA irradiated in aqueous solution against base release (Zheng et al. 1988), *G*(base release) follows the equation $G_0/G_p = 1 + \beta[\mathrm{RSH}]$. The data, compiled in Table 12.16, have been interpreted in terms of electrostatic interaction of thiols of the thiols/disulfides with DNA leading to higher concentrations of cations with respect to neutral thiols/disulfides

(and lower concentrations of anions) near DNA which allow them to scavenge •OH (thiols and disulfides) and repair DNA radicals (thiols), that is, the intermediates responsible for base release.



WR 1065



WR 33278

The effective charge *Z* of the disulfide derived from WR 1065, WR 33274, is near +4 and hence accumulates even more strongly near the DNA accounting for very effective •OH scavenging. In addition, it protects DNA by compaction (Savoye et al. 1997). It is not homogeneously distributed along the DNA double helix. As a consequence, there are hot spots of protected and unprotected regions.

Metallothioneins are small ubiquitous oligopeptides containing a high proportion of cysteine residues but no disulfide bonds (Tsunoo et al. 1978; Suzuki and Maitani 1983). Mammalian metallothioneins (MT-1 and MT-2) are made up of 61 amino acids, 20 of which are cysteine (Hamer 1986). In vivo, they participate in the absorption, storage and homeostasis of essential trace metals such as Zn and Cu (Hamer 1986; Bremner 1987; Kägi and Schäffer 1988; Stillman et al. 1992; Sato and Bremner 1993; Suzuki et al. 1993). In SV79 cells, they are reported to exert some protective effect (Greenstock et al. 1987; Abel and de Ruiter 1989). Their reaction with •OH is remarkably high (k = (1.2-3.5) × 10^{12} dm^3 mol^{-1} s^{-1}; Thornalley and Vasak 1985; Fang et al. 1994), but this high rate constant does not imply a protective role of the metallothioneins in the cellular environment against •OH (Chap. 3.5).

In all of these reactions, thiyl radicals are formed which are still quite reactive in many aspects (Chap. 7.4). In a cellular environment, they will have to be inactivated. Originally, $O_2^{\bullet -}$ was thought to be the radical sink according to reactions (78) and (79), but more recent kinetic evidence has shown that it may be the ascorbate radical [reaction (80)] (Wardman 1995, 1998).

$$RS^{\bullet} + RS^{-} \rightarrow RSSR^{\bullet -} \tag{78}$$

$$RSSR^{\bullet -} + O_2 \rightarrow RSSR + O_2^{\bullet -} \tag{79}$$

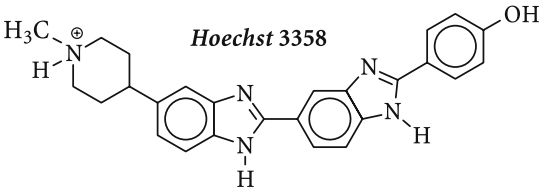
$$RS^{\bullet} + Asc^{-} \rightarrow RS^{-} + Asc^{\bullet -} \tag{80}$$

12.11.3 Bisbenzimidazoles

Bisbenzimidazoles (for a review, see Martin 1998) protect cells against ionizing radiation (Denison et al. 1992; Lyubimova et al. 2001). Bisbenzimidazoles such as *Hoechst 33258* strongly bind to the minor groove of B DNA (Pjura et al. 1987; in a dodecamer the ATTC region is the preferred intercalating site).

Table 12.17. Rate constants (unit: $dm^3\ mol^{-1}\ s^{-1}$) of the reaction of *Hoechst* 3342 with some nucleotide-derived radicals. (Adhikary et al. 1997b)

Radical	Rate constant
CMP-derived peroxyl radicals	7×10^6
TMP-derived peroxyl radicals	9×10^6
GMP-derived radicals	1.7×10^9
AMP-derived radicals	2.1×10^9



The binding kinetics and equilibrium constants for five different $(A/T)_4$ DNA sites have been studied in detail, showing that the binding kinetics are complex (Breusegem et al. 2002). Further information as to *Hoechst* binding by DNA has been obtained by fluorescence spectroscopy (Adhikary et al. 2003) and, to some extent, by atomic force microscopy (Zareie et al. 1998).

In aqueous solution, intercalated *Hoechst* 3342 protects DNA against strand breakage beyond $^{\bullet}OH$-scavenging (quenching diameter by *Hoechst* 3–4 bp; Adhikary et al. 1997a). Besides, intercalated *Hoechst* reacts with $^{\bullet}OH$-induced DNA on the ms time scale. The *Hoechst*-reactive damaged DNA sites are likely the oxidizing radicals $A^{\bullet}$ and $G^{\bullet}$ and pyrimidine peroxyl radicals (Table 12.17; for further pulse radiolysis studies on *Hoechst* in aqueous solution see Adhikary et al. 2000).

12.11.4 Small Proteins and Polyamines

Various reasons have been put forward as to why the spore is less sensitive to H_2O_2 than the vegetative cell. In the spore, the DNA is covered by the so-called small acid-soluble proteins (SASP) instead of water which makes it relatively inaccessible to oxidative damage as these SASPs may provide compaction besides sacrificial protection (e.g., Setlow 1994, 1995; Setlow and Setlow 1993).

Polyamines are ubiquitous cell components essential for normal growth and may be targets for therapeutic intervention (Marton and Pegg 1995). The protecting effects of polyamines is even more pronounced than that of proteins, such as, for example, the bacterial MC1 protein or the *lac* suppressor to their binding sites (Isabelle et al. 1993, 1999). Multiply protonated polyamines such

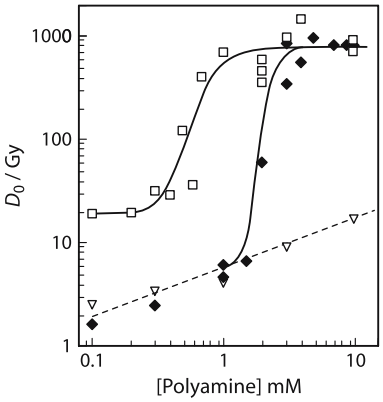


Fig. 12.15. Effects of polyamines on the •OH-induced strand-breakage of SV40 DNA (putrescine: *open triangles*, spermine: *filled diamonds*) and SV40 minichromosomes (spermine: *open squares*) according to Newton et al. (1996, with permission)

as spermine (+4) and spermidine (+3), but not the smaller putrescine (+2) (for binding constants see Braunlin et al. 1982) produce condensation of DNA and chromatin (e.g., Newton et al. 1996).

$$H_3\overset{\oplus}{N}-CH_2-CH_2-CH_2-CH_2-\overset{\oplus}{N}H_3$$

Putrescine PUT

$$H_3\overset{\oplus}{N}-CH_2-CH_2-CH_2-\overset{\oplus}{N}H_2-CH_2-CH_2-CH_2-CH_2-\overset{\oplus}{N}H_2-CH_2-CH_2-CH_2-\overset{\oplus}{N}H_3$$

Spermine SPM

This polyamine-induced compaction and aggregation of DNA (PICA effect; Gosule and Schellman 1976) leads to a protection of DNA against •OH-attack much beyond that expected on account of the •OH-scavenging capacity of the polyamines (k(•OH + putrescine) = 7×10^9 dm^3 mol^{-1} s^{-1}, k(•OH + spermine) = 1.2×10^9 dm^3 mol^{-1} s^{-1}; Newton et al. 1996; Douki et al. 2000). Putrescine with only two positive charges cannot compact DNA and thus does not provide radiation protection beyond its •OH scavenging capacity (Spotheim-Maurizot et al. 1995a; Newton et al. 1996; Fig. 12.15).

Similarly, experiments with SV40 DNA and SV40 minichromosomes (SV40 DNA covered with histones; Oudet et al. 1989) have also shown a dramatic protective effect upon neutralization of the negatively-charged DNA by the protonated polyamines, and DNA compaction (Fig. 12.15). In the SV40 minichromosomes, this occurs already at a lower spermine concentration, because the DNA surface charge is already partially compensated by the histones. In SV40 DNA, the protective effect on going from 1×10^{-3} mol dm^{-3} to 4-9 $\times 10^{-3}$ mol dm^{-3} spermidine is as much as 145 (Fig. 12.15). The higher steady-state level of damaged bases in mitochondrial DNA as compared to nuclear DNA has, in part, been

attributed to the fact that mitochondrial DNA is deficient in histones that provide protection in nuclear DNA (Eaton and Qian 2002).

DNA compaction decreases the surface area per molecule exposed to bulk •OH by 14%. Based on the cylindrical model (Mark et al. 1989), it has been calculated that competitive scavenging leads to a protection factor of 2, while a factor of 50 appears as a consequence of compaction (Newton et al. 1996). There is also a ~20% contribution by the direct effect of ionizing radiation on DNA in this special system.

Intercalation of ethidium bromide into a supercoiled plasmid *increases* its target volume at low ethidium bromide concentrations. As a consequence, low ethidium bromide concentrations sensitize plasmid DNA despite some •OH-scavenging by the drug (Begusova et al. 2000a). In linear DNA 80 bp fragments, however, the volume expansion is overcompensated by •OH-scavenging of the drug, and only protection is observed.

Since in this section compaction of DNA by spermidine has been discussed, it may be worth mentioning that compaction by spermidine has also an effect on the photochemistry of DNA. While in non-compacted DNA besides the *cis-syn* pyrimidine dimer which requires B-form DNA the formation of some *trans-syn* pyrimidine dimer is always observed, but when the DNA is compacted with spermidine, the formation of the latter is practically fully suppressed (Douki et al. 2004).

12.11.5 Chromatin

When chromatin is γ-irradiated as an expanded gel, 6 eV are required to induce an ALS (+ frank SSB) (Mee et al. 1978). This value has to be compared with 80 eV per ALS (+ frank SSB) in the whole cell. Obviously, in the expanded chromatin gel the indirect effect of radiation is much more pronounced than in the cellular environment. With DSBs as the measured endpoint, deproteinized DNA in agarose plugs (i) was compared with DNA organized in the basic nucleosome repeat (ii) condensation of the chromatin fiber into higher order structure (iii), and DNA in CHO cells (iv), an increasing protection observed (protection factor (i)→(ii) = 2; (ii)→(iii) = 8.3; (iii)→(iv) = 4.5; Warters and Lyons 1992). Thus, a protection factor near 70 is observed on going from DNA in a gel to the well protected and condensed DNA in the nucleus. As a consequence, cellular DNA base damage induced upon whole body γ-irradiation of mice are only detectable at high doses (Mori et al. 1993).

12.12 The 'Oxygen Effect', Chemical Repair and Sensitization

12.12.1 General Remarks

Free-radical-induced DNA damage is modified by dissolved O_2 or cellular components such as thiols. The latter may act beyond scavenging radicals attacking DNA, a subject that has been discussed above. The reduction of DNA radicals by thiols is termed 'chemical repair'. Yet, as has been pointed out in Chap. 11.2, this reduction does not necessarily lead to a restoration of the integrity of DNA (for the formation of α-anomers upon the reduction of the *C*(1′) radical within a dsODN see Hwang and Greenberg 1999), but this modified DNA could possibly be repaired by repair enzymes more effectively, and cell survival is enhanced (for another suggestion see below). The background of most of the studies that are reported below are attempts to improve the irradiation regimes in radiotherapy by a better understanding the underlying principles with the goal to develop drugs that enhance the radiosensitivity of tumor cell or enhance the radioresistance of normal cells. Solid tumors are often hypoxic in their center, and since hypoxic cells are less radiosensitive, these areas are likely to survive radiotherapy allowing the tumor to regrow. This reasoning has led to the search of sensitizers directed to hypoxic cells.

There is a vast literature on the response of cellular systems, from bacteria to mammalian cells, but also animal and even clinical studies. In the present context, the emphasis will be on mechanistic details concerning the free-radical aspects. The most important late effects, notably the processing of DNA damage by the repair enzymes cannot be dealt with here.

12.12.2 Oxygen Effect and Chemical Repair

A marked effect of O_2, as we know now, on the response of ionizing radiation on tissue was first observed by Schwarz (1909). He had exposed the skin to a radium probe and observed that an erythema development more slowly when the probe was tightly clamped to the arm of a patient. Such phenomena are now called 'oxygen effect' (for an early detailed report, see Gray 1954), for a historical review, see Hall 1982). Typically, a given cell population is inactivated in the presence of O_2 about three times as effectively as under anoxic conditions (oxygen enhancement ratio: OER = 3). On this basis, the importance of O_2 in radiotherapy has been addressed (Gray et al. 1953). As a mechanistic explanation of this phenomenon, it has been suggested that thiols (RSH) could repair DNA damage ('chemical repair'; Alexander and Charlesby 1955) which is otherwise fixed by O_2 (Ormerod and Alexander 1963).

The OER is expressed by the Alper formula (81), where m is the maximum response at very high $[O_2]$ and K is the $[O_2]$ when the response is half of its maximum value (Alper 1979).

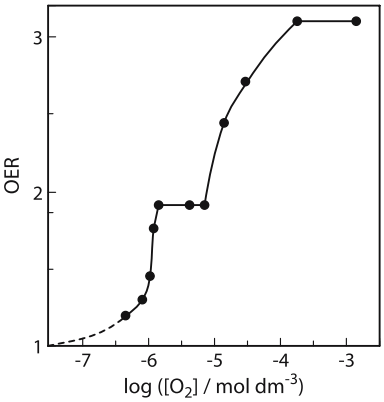


Fig. 12.16. OER for the survival of V 79-135B cells as a function of the O_2 concentration in the medium. Data taken from Millar et al. (1979)

$$\text{OER} = (m\,[\text{O}_2] + K) / ([\text{O}_2] + K) \tag{81}$$

The concept is based on a very simplified scheme [reactions (82)-(86)].

$$\text{DNA} + \text{ionizing radiation} \rightarrow \text{damaged DNA} \tag{82}$$

$$\text{DNA} + \text{ionizing radiation} \rightarrow \text{DNA}^{\bullet}\ (\text{various DNA radicals}) \tag{83}$$

$$\text{DNA}^{\bullet} \rightarrow \text{damaged DNA} \tag{84}$$

$$\text{DNA}^{\bullet} + \text{RSH} \rightarrow \text{DNA}\ (\text{chemical repair}) \tag{85}$$

$$\text{DNA}^{\bullet} + \text{O}_2 \rightarrow \text{DNA-O}_2^{\bullet}\ (\text{oxygen fixation}) \tag{86}$$

The importance of reaction (84) in this scheme has been pointed out (Schulte-Frohlinde and Bothe 1990), and on the model level, transforming DNA (Held et al. 1981) or SSB formation (Prakash Rao et al. 1992), a chemical repair by RSH beyond $^{\bullet}$OH-scavenging [reaction (85)] has been clearly shown.

However, this simplified scheme does not account for the fact that in many cases the oxygen effect is biphasic or even more complex (e.g., Ewing and Powers1976, 1980; Millar et al. 1979; Tallentire et al. 1972), and the mathematics of such a complex situation has been discussed (Millar and Scott 1981; Alper 1983, 1984; Koch 1985; Scott 1986). An example of such a multiphasic OER is shown in Fig. 12.16.

The time scale of the oxygen effect is of particular interest as it yields information as to the lifetime of the free-radical lesions in a cellular environment. Several approaches to measure such lifetimes have been followed (Adams et al. 1976b; Michael 1984; Adams 1985).

In the double-pulse method, the first pulse directed on wet cells consumes, by free-radical reactions, all the O_2 which is dissolved in the cells. The second pulse

Table 12.18. First half-life ($t_{½}$) of the decay of the O_2-dependent damage to various strains of *E. coli* K 14. (Michael et al. 1981b)

Strain	Relevant phenotype	$t_{½}$/ms
1157	Wild-type	0.2
830	GSH-synthetase-deficient	0.45
821	γ-Glutamyl-synthetase-deficient	2.6
7	γ-Glutamyl-synthetase-deficient	1.6

is given after a certain time which allows the cells to re-oxygenate. The amount of O_2 re-diffused to the critical targets within the cell during the inter-pulse time is then inferred from the known response of cells to single short pulses of radiation (Epp et al. 1973, 1976; Ling et al. 1978; Michaels et al. 1981a, b).

In the oxygen explosion technique (Chap. 13.4), an electron pulse is delivered to a thin layer of anoxic cells to which O_2 is admitted in a flash either before or after the pulse (Michael et al. 1973, 1979).

Another approach uses a rapid-mixing device. One of the solutions contains the cells in a deoxygenated nutrient broth, while the other contains an appropriate amount of O_2 (Shenoy et al. 1975; Whillans and Hunt 1978, 1982; an extension of this method is the rapid-lysis technique; Fox et al. 1976; Tilby and Loverock 1983).

With *S. marescens*, the rate constant for the reaction of O_2 with the target radical has been calculated at 5×10^8 dm^3 mol^{-1} s^{-1} (Michael et al. 1981b). This reaction is somewhat slower than the typical rate of reaction of O_2 with free-radicals in aqueous solution (~2×10^9 dm^3 mol^{-1} s^{-1}; Chap. 8.2), but the environment around the target radicals may be considerably more viscous and the O_2 solubility lower (Chap. 8.1), and these factors may contribute to the lower rate.

In cells, the major contributor to the thiol pool is glutathione (GSH); Quintiliani 1983; for the cellular content of low-molecular-weight thiols see Fahey and Newton 1983; for their determination see Fahey and Newton 1987). In mammalian cells its distribution is not homogeneous, but a typical value is (1-2) $\times 10^{-3}$ mol dm^{-3} (Wardman and von Sonntag 1995; for the determination of biologically relevant thiols see Fenton and Fahey 1986; for the low-molecular-weight thiol content in some microorganisms see Fahey et al. 1987). The synthesis of GSH requires the enzyme glutathione synthetase, and there are a number of mutants that lack this enzyme and consequently are deficient in GSH. Such mutants have been used to explore details of chemical repair and the oxygen effect (Edgren et al. 1980, 1984, 1985; Edgren 1982; Deschavanne et al. 1985; Edgren and Revesz 1985; Debieu et al. 1985; Midander et al. 1986; for a review, see Revesz 1985).

The first half-lives of the decay of the O_2-dependent damage to various strains of *E. coli* K 40 are compiled in Table 12.18. Compared to the wild-type, the other

Table 12.19. Chemical repair rates for O_2-dependent free-radical precursors of lesions in V70 cells measured using the gas explosion technique. (Prise et al. 1992)

	Rate/s^{-1}	$t_{½}$/ms
Cell survival	530 ± 210	1.3
DSB (pH 9.6)	380 ± 180	1.8
DSB (pH 7.6)	670 ± 360	1.0
SSB	210 ± 85	3.4

Table 12.20. Second-order rate constants (unit: $dm^3\ mol^{-1}\ s^{-1}$) for fixation by O_2 and chemical repair by GSH of SSB and DSB free-radical precursors. (Prise et al. 1999)

	SSBs	DSBs
O_2	$(2.3 \pm 0.02) \times 10^8$	$(8.9 \pm 1.2) \times 10^7$
GSH	$(1.4 \pm 0.3) \times 10^5$	$(2.9 \pm 0.4) \times 10^5$

strains contain less of GSH. Since fixation and chemical repair are competing processes, a faster repair would also result in a shorter lifetime of the O_2-dependent damage. This being observed, the data support the above concept.

With pBR 322 plasmid DNA in aqueous solution containing 0.01 mol dm^{-3} GSH and using the gas explosion technique, the rate of repair of SSB precursors was determined at 1370 s^{-1} as compared to 2900 s^{-1} for DSB precursors (Prise et al. 1993). The OER for SSB was 3.0 and that for DSB 7.5. This is strong evidence that in this model system DSBs are formed by two close-by SSBs.

The chemical repair rates for the survival of V70 cells and two typical lesions, DSBs and SSBs are shown in Table 12.19. There is no difference between cell survival and DSB formation when the large error bars are taken into account. In contrast, the repair rate for SSBs is much slower. This difference may be accounted for on the basis of the clustered lesion model (Sect. 12.5) considering that if one of two precursors of nearby SSBs has been successfully repaired, a DSB can no longer develop. Note that the probability of one or the other of these radicals being chemically repaired is twice that of a that of an SSB free-radical precursor.

Such studies have been extended to plasmid pBR 322 DNA by modifying the gas explosion technique and using H_2S as a repair agent (Prise et al. 1998). With this assay, it was shown that the post-irradiation protection of DNA by H_2S is time-dependent, having first-order rate constants of 21 s^{-1} for SSB and 10 s^{-1} for

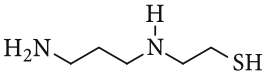
Table 12.21. Second-order rate constants for thiols added to GSH-deficient *E. coli*. (Prise et al. 1995; Fahey et al. 1991)

Thiol	Net charge *Z*	k/dm^3 mol^{-1} s^{-1}	
		E. coli	pBR 322
GSH	–1	$(1.2 \pm 0.3) \times 10^5$	$(2.1 \pm 0.1) \times 10^5$
3-Mercaptopropionic acid	–1	$(1.3 \pm 0.6) \times 10^5$	n.d.
2-Mercaptoethanol	0	$(3.3 \pm 1.6) \times 10^5$	$(1.4 \pm 0.6) \times 10^6$
Cysteamine	+1	$(3.9 \pm 1.1) \times 10^5$	$(1.2 \pm 0.3) \times 10^7$
WR 1065	+2	$(2.7 \pm 1.1) \times 10^6$	$(6.6 \pm 2.0) \times 10^7$

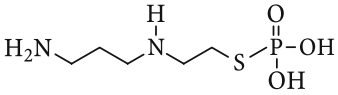
n.d., Not determined

DSB formation. These data and the kinetic parameters shown in Table 12.20 further support the view that DSBs are indeed formed by two close-by SSBs.

It has been shown above that there is a marked effect of the charge of the thiol and its efficiency to protect against •OH attack but also to repair DNA damage (for thiol binding to DNA see Smoluk et al. 1986). An extension to GSH-deficient cells in combination with the oxygen explosion technique allowed to determine the rate constants of various thiols with radiation-induced DNA damage (Table 12.21; Prise et al. 1995).



WR 1065



WR 2721

In aqueous solution around pH 7, WR 1065 is doubly protonated (net charge $Z = 2$). Its uptake by mammalian cells is kinetically of first-order and increases with $[H^+]^{-1/2}$. This has been taken as evidence that the transport through plasma membrane probably occurs by a passive diffusion of the uncharged diamine (Calabro-Jones et al. 1988).

The WR 1065 derivative WR 2721 has found considerable attention because it has been reported to be capable of selectively protecting normal vs malignant cells (Phillips 1980; Yuhas 1982). To convert WR 2721 into the reactive protector WR 1065, a phosphatase is required (Calabro-Jones et al. 1985; Smoluk et al. 1988a; Purdie 1980). The lower pH in tumors and concomitant slower rate of drug uptake combined with their lower content of phosphatases has been suggested to be the basis of this selective protection (for the use of WR 2721 in the clinic see Hospers et al. 1999). Methods for the detection of these drugs in plasma have been developed (Fahey and Newton 1985), and some rate constants

Table 12.22. Non-protein sulfhydryl (NPSH) and glutathione (GSH) content (μmol/mg protein) of normal ($GSH^{+/+}$) and GSH-deficient ($GSH^{-/-}$) mammalian cells and the radiation response of these cells. (Revesz 1985)

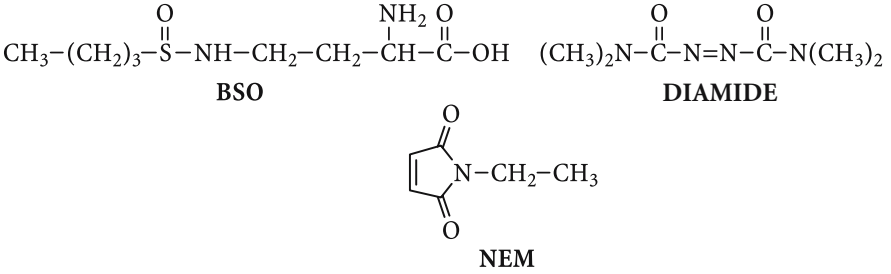
	$GSH^{+/+}$	$GSH^{-/-}$
NPSH	12.9	6.0
GSH	11.6	0.7
OER of SSB	3.0	1.2
OER of survival	2.9	1.5
OER of survival, Cya 1×10^{-3} mol dm^{-3}	3.2	1.8
OER of survival, Cya 20×10^{-3} mol dm^{-3}	4.1	2.3
MER of SSB	1.9	1.0
MER of survival	2.3	1.5

OER, Oxygen enhancement ratio; MER misonidazole enhancement ration; Cya, cysteamine

of WR 1065 relevant for $^{\bullet}OH$-scavenging and repair were determined by pulse radiolysis (Ward and Mora-Arellano 1984). The use of these drugs seems not to be without hazard, since with WR 1065 selective denaturation of nonhistone nuclear proteins is observed (Botth et al. 2000). It also retards the rate of rejoining SSBs (Murray et al. 1988a, b).

The radiation response of cells from a GSH-synthetase-deficient patient and its clinically healthy brother are compared in Table 12.22. There, it is seen that the GSH deficiency results in a lower OER both of survival and SSB formation. This is largely due to a reduction of GSH, since the other non-protein sulfhydryls (NPSH) are not markedly suppressed in this mutant. The efficiency of GSH has been attributed to its particular spatial distribution, since exogenous thiols (here: cysteamine) cannot substitute for this. Other sensitizers such as misonidazole (see below) behave similar as O_2 with respect to SSB formation in these two cell lines.

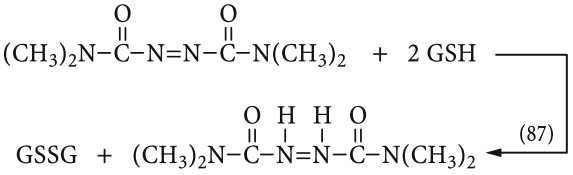
As one might expect from the above, there is a correlation between the OER and the NPSH content in mammalian cells (Cullen et al. 1980). The NPSH level may be reduced by the addition of several drugs such as BSO, DIAMIDE or NEM (for reviews see Bridges 1969; Biaglow et al. 1983; Shenoy and Singh 1985).



BSO **DIAMIDE**

NEM

While BSO interferes with the biosynthesis of GSH (Biaglow et al. 1983; Romero and Sies 1984), DIAMIDE oxidizes GSH to the disulfide [reaction (87); Harris 1982]. It has also been considered to act as a sensitizer by preventing low O_2 concentrations from being depleted at high dose rates (Clark et al. 1983).



(87)

NEM and also diethylmaleate (DEM) reduce the GSH content of cells by binding (Bump et al. 1982), but other reaction pathways have been considered as well (Mullenger and Ormerod 1969). NEM, for example, is also effective when given shortly after irradiation (Han et al. 1976) pointing to an interference with the enzymatic repair rather than at the level of free-radical reactions. The reactions with DNA radicals is too slow to be of any marked effect (Simic and Hayon 1971; Hayon and Simic 1972).

Some radicals are sluggishly reduced by thiols but very fast by thiolates (Chap. 7.4). The fraction of GSH present in the thiolate form (GS^-) [pK_a(GSH) ≈ 9.2; Wardman and von Sonntag 1995] is given by Eq. (88).

$$[GS^-]/(GSH] + [GS^-] = [1 + 10^{(pKa - pH)}]^{-1} \quad (88)$$

Thus at pH = 7.4, ~1.6% of the total GSH is in the ionized form. This concentration is high enough for some radicals to be reduced by GS^- in equilibrium rather than by GSH.

Instead of reducing the cellular GSH level, it may also enhanced upon the addition of the drug OTZ which stimulates GSH synthesis thereby approximately doubling the cellular GSH content. At low O_2 concentrations, this leads to an increased protection (Russo et al. 1985).

Although there is such convincing evidence as to the involvement of thiols on the oxygen effect, an intriguing problem remains. In their reactions with thiols, the integrity of DNA is rarely restored, but, taking an $^{\bullet}OH$-base adduct as an example, the resulting product formed by H-donation is a base hydrate (Chap. 11.2). This is a DNA lesion that has to undergo repair as other DNA lesions. One may argue that this type of damage is easier to repair than O_2-mediated damage.

Table 12.23. Reduction potentials of some amino acids at pH 7; data taken from Milligan et al. (2003)

Amino acid	Reduction potential/V (vs NHE)
Cysteine	+ 0.92
Cystine	+ 1.1
Histidine	+ 1.17
Methionine	+ 1.5
Tryptophan	+ 1.03
Tyrosine	+ 0.93

This has been put forward as a possibility in many discussions for some time. Yet, it is not a very convincing possibility. In recent years, the importance of the complexity of DNA damage for an inadequate repair has been become apparent. Clustered lesions increase the complexity of DNA damage, and most clustered lesions involve O_2. It is tempting to suggest that thiols interrupt at an early stage the formation of clustered lesions and thus reduce the complexity of DNA damage. It would be a major breakthrough in our understanding of the oxygen effect if this question could be addressed experimentally.

DNA is typically surrounded by proteins, and the question obviously arises as to what extent they may contribute to a repair of DNA radicals. The $G^{\bullet+}/G^{\bullet}$ radical has been generated in plasmid DNA in aqueous solution with the help of $(SCN)_2^{\bullet-}$, and the formation of G lesions has been monitored as SSBs induced by the Fpg protein (Milligan et al. 2003). The repair of $G^{\bullet+}/G^{\bullet}$ by electron-donating amino acids has been studied. Most of these, notably tryptophan, were found to be quite reactive except for histidine and some derivatives of cystine and methionine (Table 12.23).

Attention has been drawn to the fact that the reduction potentials of the amino acids in proteins may differ by –0.2 and +0.3 V from the above values, and this may also influence the repair capability of proteins surrounding the DNA. ET in DNA and proteins over a longer distance have been discussed above.

The phenolic catechins found in green tea are related to the phenol tyrosine, and it hence not surprising that they also can repair some of the radical-induced DNA damage in aqueous solution (Anderson et al. 2001). For being of any consequence for DNA repair in vivo (as sometimes suggested), they would have to accumulate near DNA, and this has not yet been shown to our knowledge.

The C-H BDE of the peptide linkage in proteins is lower than the RS-H BDE (Chap. 7.4), and thermodynamically a reduction (by H-donation) of DNA radicals by surrounding proteins is favored over a reduction by GSH and other thiols. Yet, such H-donation reactions by peptides are kinetically disfavored. As a

Table 12.24. RBE and OER values for cell inactivation and induction of DSBs in V79-4 cells on the photon energy of the ultrasoft X rays. (de Lara et al. 2001)

		RBE		OER	
Radiation type	**Energy**	**Survival (10%)**	**DSBs**	**Survival (10%)**	**DSBs**
^{60}Co-γ rays	1.17/1.33 MeV	1	1	2.8	3.5
Ti K-shell X rays	4.55 keV	1.5	1.4	2.0	1.9
Al K-shell X rays	1.49 keV	1.7	1.9	1.9	2.1
Cu L-shell X rays	0.96	2.3	2.3	2.0	1.8
C K-shell X rays	0.28 keV	2.8	2.7	2.2	1.8

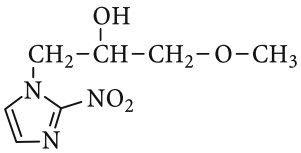
consequence, the reaction is comparatively slow and may become effective only under certain conditions. For example, one may envisage that the DNA radical happens to be in quasi-contact with the peptide C-H of a surrounding protein and the latter shields the DNA radical from an approach by O_2 or GSH. The lifetime of the O_2-dependent DNA damage has been given as 0.2 ms in Table 12.18. Taking the 'peptide concentration' as 10 mol dm^{-3} (i.e. a quasi-solvation of the DNA radical by peptide C-H groups of the surrounding protein), one would arrive at a rate constant of only 3.5×10^2 dm^3 mol^{-1} s^{-1} to become competitive. According to the data given for the *lac* suppressor-*lac* operator complex (cf. Begusova et al. 2001a) at least one peptide hydrogen may came close enough (3.45 Å, near ≤ 3.4 Å the crystallographic distance, where H-transfer has been observed in chain reactions passing through carbohydrate crystals such as 2-deoxyribose; Schuchmann et al. 1981 and D-fructose; Dizdaroglu et al. 1976). Yet, the observed protection at the given position can be adequately described by the RADACK procedure (Sect. 12.2.1), and there is not yet experimental evidence for protection and repair beyond radical scavenging and induction of conformational DNA changes.

As the LET of the ionizing radiation increases, the RBE increases as do clustered lesions such as DSBs. The presence of O_2 has then no longer such a dominating effect on increasing the complexity of DNA lesions and thus the OER drops. This is exemplified for ultrasoft X-rays in Table 12.24.

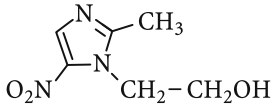
12.12.3 Sensitization

It has been pointed our above that solid tumors may have a hypoxic core (for the detection of hypoxic regions see Porschen et al. 1977; Chapman 1984), and that it would be highly desirable to sensitize these areas with a drug that is not as rapidly metabolized as O_2 and therefore can reach the site of action and mimic O_2 with respect to its capability to fix DNA damage. Many of the drugs that have been

studied contain nitro groups (for reviews see Adams et al. 1974, 1979; Adams 1977; Wardman 1977, 1984; Wardman and Clarke 1985; Durand and Olive 1981). Typical examples are the nitroamidazoles misonidazole and metronidazole ('Flagyl') which is most commonly used in the clinic (Wardman et al. 2003).



Misonidazole



Metronidazole ('Flagyl')

Nitro compounds may add to carbon-centered radicals and thus also with the majority of the DNA radicals (Chap. 6.3; only the very strongly reducing radicals such as e_{aq}^- and $CO_2^{\bullet -}$ reduce the nitro sensitizers to (unstable) hydroxylamines; McClelland et al. 1984). Originally, the nitro compounds and O_2 have just been taken as 'oxidants' irrespective of their mode of action, especially as the efficiency of the sensitizers correlates with their reduction potential (Adams and Cooke 1969; Tallentire et al. 1972; Simic and Powers 1974; Adams et al. 1976a, 1981). This concept is expressed in relationship (88), where C is the sensitizer concentration required to achieve a constant sensitizing response (e.g. an enhancement ratio of 1.6) and E^7 the one-electron reduction potential of the sensitizer at pH 7.

$$- \log C = b_0 + b_1 E^7 \tag{89}$$

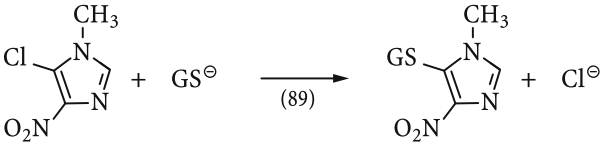
The electron affinity relationship (Table 12.25) does not uniquely apply to radiation sensitization but also, for example, to toxicity (Olive 1980), and a warning was expressed against drawing a firm conclusion from this relationship as to the cause of the effect (Wardman and Clarke 1985).

In fact, with a few exceptions, neither O_2 nor nitro compounds react with free radicals by ET (as would be required for an electron affinity relationship in its proper sense) but rather undergo addition reactions. The ensuing radicals show different decay routes as is discussed in Chapter 6.3. When DNA is irradiated in deoxygenated aqueous solution in the presence of ^{14}C-labeled mitronidazole, some of the sensitizer remains attached to DNA (Willson et al. 1974). Misonidazole and practically all other nitroimidazoles have a lower reduction potential than O_2, and much higher concentrations have to administered to achieve the same effect (Whillans and Hunt 1982).

Some of the sensitizing (and cytotoxic) effects of the nitro compounds can be attributed to GSH depletion. Activated chlorine may be replaced by GS^- [reaction (89); Wardman 1982; Stratford et al. 1983)], but the reaction is much more general. For example, the 2-nitroimidazoles react with GS^- by NO_2^- release. These reactions are slow, but are considerably speeded up by glutathione-*S*-transferase (Wardman et al. 1973).

Table 12.25. Some electron affinity relationships with nitro compounds compiled by Durand and Olive (1981)

Property or reaction	Number of studies	Redox dependence (b_1/V^{-1})	
		Mean	Range
Radiosensitization	3	9.5	6.0–10.8
Reduction by			
Aerobic bacteria	1	8.2	
Anaerobic mammalian cells	1	10.7	
Anaerobic microsomes	1	10.5	
$FMNH_2$	1	18.4	
Xanthine/xanthine oxidase	1	13.8	
Cytotoxicity			
Bacteria	2	101.5	11.4–11.7
Anaerobic mammalian cells	1	7.4	
Aerobic mammalian cells	3	8.7	8.4–9.0
Mutagenicity			
Aerobic bacteria	3	11.2	9.5–12.3
Anaerobic mammalian cells	1	7.4	
DNA			
Synthesis	1	12.5	
Strand breakage	1	9.8	
Release of Thd by reduced nitroimidazoles	1	11	



It may be mentioned that to study the effects of sensitizers on DNA radicals by γ-irradiating biologically active DNA in aqueous solution in the presence of sensitizers does not provide useful information, since under such conditions the sensitizers scavenge •OH and thus protect rather than sensitize (Lafleur and Loman 1982; for •OH-scavenging see Whillans et al. 1975).

The actual use of nitro compounds such as misonidazole in cancer treatment is largely based on their toxicity against hypoxic cells. Here, they act as biore-

ductive drugs (Wardman et al. 2003; Wardman 2001). The damage to DNA may not be via free radicals in this case, but due to a reaction of the hydroxylamines (formed upon the reduction of the nitro compounds) with G (for references see Wardman et al. 2003).

Sensitization of anoxic cells is also brought about by non-toxic concentrations of transition metal ions (< some 10^{-4} mol dm^{-3}) such as Cu(I). A dose-modifying factor of ~1.5 (at 6.6×10^{-5} mol dm^{-3} Cu(I)) has been observed for mammalian cells (Hesslewood et al. 1978), but no sensitizing effects were observed for oxygenated cells. Under anoxic conditions, reduction of Cu(II) to Cu(I) occurs within the cells without an added reductant (see also Cramp 1967). It would be premature to come up with detailled mechanistic concepts, but some aspects of the actions of transition-metal ions have been discussed in Chapter 2.5.

12.13 Radiolysis of DNA in the Solid State

The various aspects of the EPR spectra of γ-irradiated DNA has been reviewed in quite some detail (Wyard and Elliott 1973; Hüttermann 1982, 1991; Becker and Sevilla 1998; Symons 1999; Close 2003; Bernhard and Close 2003). The main feature is the formation of electron gain centers at the Thy and Cyt moieties (for some difficulties in discerning among these two see Cullis et al. 1992), while the hole is largely located at Gua. The radicals at sugar moiety usually escape identification and the reason for this has been discussed (Close 1999). The radical yield as detected by EPR drops dramatically with increasing LET (Sevilla et al. 2000; for a review on the recombination of radicals in solid DNA, see Bernhard et al. 1994). Yet, at high LET (Ar ion beam), the yields of the charged base radicals are reduced and radicals located at the sugar moiety and at the phosphorus are detectable (Becker et al. 2003). They have been attributed to the scavenging of low-energy electrons (Chap. 4.3) that have been shown to cause resonant formation of DNA strand breaks (Boudaiffa et al. 2000a, 2002; for higher electron energies see Boudaiffa et al. 2000b; Huels et al. 2003) and scission of the glycosidic linkage (as shown for Thd; Zheng et al. 2004).

In the presence of Tl^{3+} ions that scavenge electrons and produce the strongly oxidizing Tl^{2+} ion (Schwarz and Dodson 1984), the yield of $G^{\bullet+}$ is increased (Shukla et al. 2004). Its reaction with water gives rise to the G *C*(8)-$^{\bullet}$OH-adduct that is subsequently oxidized to 8-oxo-G. Hole transfer through DNA (Sect. 12.10) results in the formation of the 8-oxo-G radical cation that is detected. With anthracycline-d(CGATCG) complexes, both the hole and the electron are scavenged by the drug, and no DNA radicals are observed by EPR (Milano et al. 1998).

Hydration of the DNA has also a strong influence on the radical yield at 77K (Wang et al. 1993). The *G* values increase by over fourfold upon addition of the primary hydration layer, that is about 20 water molecules per nucleotide. Upon further water addition, the excess water freezes into an apparently independent bulk ice phase which "steals" about five water molecules from the hydration layer and thus reduces the DNA radical yield. It has been concluded that efficient hole

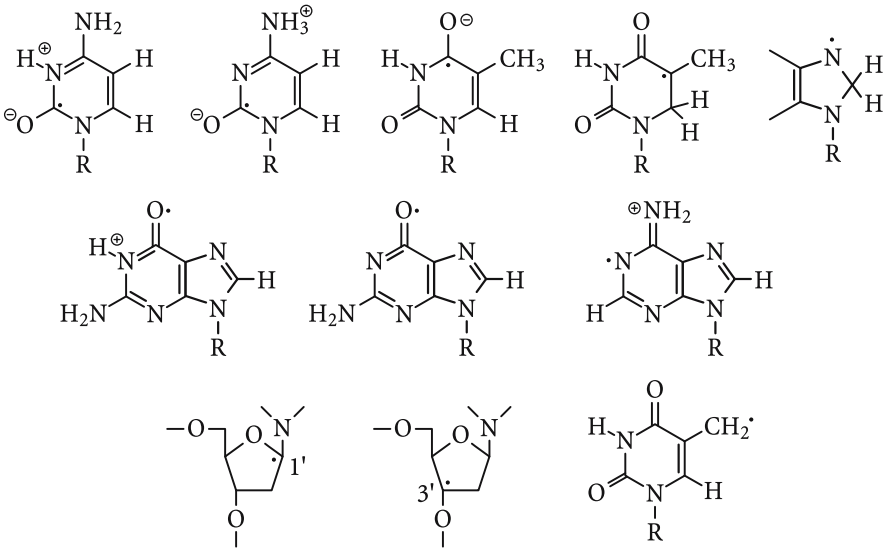


Fig. 12.17. Radical structures assigned in γ-irradiated solid DNA

and ET to DNA occurs from the hydration shell. Some •OH are also formed and trapped in this hydration layer (Becker et al. 1994; Mroczka and Bernhard 1993) and may be scavenged by a spin trap upon warming up (Ohshima et al. 1996; La Vere et al. 1996). In dry DNA, T and C, and to some extent A, are the trapping sites for the electron and G for the hole (Wang et al. 1994; see also Sevilla et al. 1991). More than ten chemical structures have been assigned (Fig. 12.17; Weiland and Hüttermann 1998; Yan et al. 1992). They mainly consist of the one-electron-reduced pyrimidines (in different states of protonation). The one-electron-reduced T converts by protonation at *C*(6) (Chap. 10.4). H atom scavenging by the purines or protonation of their electron adducts at *C*(8) yields an *N*-centered radical. The purines largely serve as the sink for the hole. Detected H-abstraction sites were attributed to the sugar moiety and the methyl group of T. The latter radical may also be produced by the deprotonation of the T radical cation (Chap. 10.2). This has been put on a quantitative basis, and it has been suggested that at early times the electrons are captured by T (30–35%) and C (20–28%), while among the radical cations G (26–28%) and A (8–17%) dominate (Yan et al. 1992). ET from $C^{\bullet-}$ to T and subsequent protonation at *C*(6) occurs at a later stage.

Upon γ-irradiation of dry DNA unaltered bases are set free with a *G* value of about 0.2×10^{-7} mol J^{-1} for Ade, Cyt and Thy, and 0.12×10^{-7} mol J^{-1} for Gua in the absence of O_2, and 0.4×10^{-7} mol J^{-1} (Ade, Cyt and Thy) and 0.25×10^{-7} mol J^{-1} (Gua) in its presence (Swarts et al. 1992). Hydration of the DNA lowers these values somewhat. The precursor of these unaltered bases are sugar radicals, i.e. largely sugar radical cations. These are strong oxidants, and since G is the best electron donor among the nucleobases, it is likely that the G-bound sugar radical cation may effectively be reduced by the neighboring G (Sect. 12.10). This must

Table 12.26. Product yields (*G* values, unit: of 10^{-7} mol J^{-1}) of differently hydrated DNA γ-irradiated under N_2. (Swarts et al. 1996)

Product	Γ = 2.5	Γ = 10.3	Γ = 13.2	Γ = 24.4	Γ = 32.8
8-oxo-G	1.0	0.81	0.84	0.72	0.69
FAPY-G	0.12	0.21	0.13	0.081	0.16
Gua	0.12	0.079	0.08	0.095	0.094
8-oxo-A	0.014	0.015	0.018	0.013	0.014
FAPY-A	0.005	0.008	0.004	0.012	0.022
2-OH-Ade	0.006	0.004	0.005	0.01	0.01
Ade	0.21	0.13	0.14	0.16	0.17
5-OH-Ura	0.014	0.007	0.003	0.011	0.016
5-OH-Cyt	0.004	0.001	0.002	0.008	0.007
5,6-diOH-Ura	0.004	0.003	0.004	0.006	0.005
5-OH-Hyd	0.019	0.008	0.004	0.002	0.003
Cyt	0.21	0.14	0.13	0.13	0.15
5,6-diOHThy	0.26	0.67	0.51	0.11	0.093
5-OHMe-Ura	0.05	0.027	0.022	0.035	0.03
5-OH-5,6diHThy	0.022	0.015	0.013	0.004	0.007
Tg	0.007	0.003	0.011	0.007	0.013
5-OH-5-Me-Hyd	0.017	0.006	0.001	0.003	0.004
Thy	0.019	0.12	0.13	0.17	0.20

lower the free Gua yield, as is indeed observed. The results of a detailed product study are compiled in Table 12.26 (for H_2 and dihydropyrimidine yields as a function of the water content see Falcone et al. 2005).

As SSBs are concerned, data are available on single crystals of dsODNs of different composition (Debije et al. 2001; Razskazovskiy et al. 2003a, 2003b). There is little base sequence specificity and preference of 5′-cleavage vs 3′-cleavage. Although there is a certain span for *G*(SSB) among the various systems studied ((~0.4-1.4) × 10^{-7} mol J^{-1}), these data clearly point to the importance of SSB formation by the direct effect.

In the presence of electron scavengers such as iodoacetamide, electron-gain centers are transformed into alkyl-type radicals which preferentially abstract a

hydrogen from the methyl group of T (Razskazovskii et al. 1998). Aryl radicals generated from intercalated *o,o′*-diphenyleneiodonium ions are less selective and abstract also hydrogens from the sugar moiety, notably *C*(1′) as well as add to the *C*(5)-*C*(6) double bond of T.

12.14 Photoionization

With low-energy photons exciting the first absorption band ($\lambda > 235$ nm), pyrimidine cyclobutane dimers and (6-4)-photoproducts dominate the product spectrum, but there are also some minor products that must result from free-radical-induced reactions (Cadet et al. 1992; von Sonntag et al. 2004a). At higher energies (for absorption cross-sections see Inagaki et al. 1974; Henke et al. 1993) photoionization is observed. In aqueous solution, for example, biphotonic excitation at 248 nm leads to photoionization and SSBs in 5% yield compared to the former (Bothe et al. 1990; for a review on the photoionization of DNA see Görner 1994). Photoionization of aqueous DNA by 193-nm light is monophotonic (Φ = 0.048–0.065; 0.02; Görner et al. 1992) and takes place mainly (>50%) at G, but additional hole transfer from other ionized nucleobases increases the yield of $G^{\bullet+}$ or its deprotonated form $G^{\bullet}$ (Melvin et al. 1995b). SSBs, although only < 2% of the photoionization yield, occur preferentially at 3′ to a G residue. Treatment with Fpg, Nth or hot piperidine shows that selective, non-random modification at G must occur (Melvin et al. 1998). The formation of low yields of pyrimidine dimers have also been monitored with the help of T4 endonuclease (T4 endo V) under these conditions, but G is the major site of damage. Using *E. coli* strains of different repair deficiencies, it has been shown that at 193-nm repair-efficient strains are mainly inactivated by the lesions set by photoionization, while pyrimidine dimers play the major role in the inactivation of strains that lack the corresponding repair enzymes (Gurzadyan et al. 1995). With 7-10-eV photons in aqueous solutions, SSBs and DSBs are largely formed via water radicals formed in the water photolysis (Folkard et al. 2002).

In the dry state, the cross-section for SSB formation in ΦX174-DNA increases by five orders of magnitude approximately continuously between 5 and 10 eV of photon energy (Wirths and Jung 1972), and only at about 12 eV the ratio of SSB per inactivation approaches unity (Jung et al. 1977; this includes 'collisions of the second kind' from excited metastable gas molecules; see also Lücke-Huhle and Jung 1973; Lücke-Huhle et al. 1974). This agrees with the observation that at 150 nm the quantum yields of pyrimidine dimer is noticeably lower than at $\lambda > 200$ nm (Yamada and Hieda 1992; the pyrimidine cyclobutane dimers to (6-4)-photoproducts also drops substantially in this wavelength region; Matsunga et al. 1991; for the inactivation of spores see Keller and Horneck 1992). The SSB quantum yield (in vacuum) is $\sim 2 \times 10^{-3}$ at 7 eV and increases to 0.3 at 150 eV (Prise et al. 2000; for some earlier data see Ito and Taniguchi 1986). The corresponding DSB yield are ~3% of these values, 6×10^{-5} at 7 eV and 0.01 at 150 eV (for the setup, see Folkard et al. 2000). The observation of low yields of prompt DSBs (besides high yields of SSBs) under such conditions has led to a similar

discussion as for the analogous phenomenon observed in the radiolysis of highly scavenged DNA solutions (Sect. 12.4.4).

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13.1 General Remarks

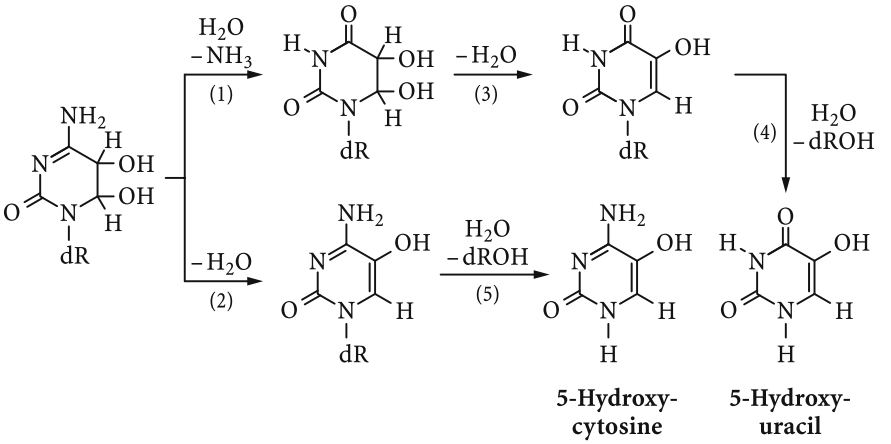
For the detection of free-radical-induced DNA damage and its repair, biochemical techniques are increasingly applied. It would exceed the allocated space to discuss these techniques here, and the reader is referred to the original literature. However, to assist biologists and biophysicists, some of the chemical techniques for measuring typical DNA lesions are discussed. Most of the kinetic data concerning the free-radical chemistry of DNA and its model systems has been obtained by pulse radiolysis. This technique is only available in a few laboratories worldwide. For this reason, it will be described in some detail here.

13.2 Detection of DNA Damage

In recent years, the sensitivity of the detection of free-radical-induced DNA damage has been considerably increased (for brief reviews, see Cadet and Weinfeld 1993; Cadet et al. 1997a, b, 1999b). There is often a problem with lesions that are refractory to an enzymatic excision (Dizdaroglu et al. 1978, and further examples are reported below), but it is expected that when enzymatic degradation techniques have been further developed, acid hydrolysis and its marked problems will no longer be required.

13.2.1 Excision of Damaged Bases by Acid Hydrolysis

Damaged DNA bases are usually still attached to the DNA backbone via a nucleosidic linkage. In order to release them for analysis, the nucleosidic linkage has to be hydrolyzed. Common procedures use 60–88% formic acid at 140 °C for 2 h. As a milder condition, 35% HF in pyridine at 37 °C for 2 h has been suggested (Douki et al. 1996) and, in particular, FAPY-A and FAPY-G withstand concomitant degradation much better (Douki et al. 1997). Nevertheless, all these conditions are very severe, and the product may be altered by this treatment. A case in point are the cytosine glycols which do not withstand this acid treatment and are released as 5OHCyt and 5OHUra [reactions (1)–(5)] (Dizdaroglu et al. 1986; Douki et al. 1996).



Although 35% HF in pyridine is considered to be milder than the formic acid treatment, HMUra is degraded under such conditions (Douki et al. 1996). Moreover, 60% formic acid is believed to be a stronger hydrolyzing agent as compared to 88% formic acid due to the higher degree of dissociation. Yet, the final yield of HMUra (in the plateau region after 2 h) is significantly lower in the case of the lower formic acid concentration (Douki et al. 1996). Thus, there is no ideal condition, and for each DNA lesion special conditions may have to be worked out to avoid major artifacts (Cadet et al. 1997a). Apparently, the discussion on potential artifacts continues (Dizdaroglu 1998) and experimental expertise acquired over the years in one laboratory is difficult to transfer to another one.

13.2.2 Excision by Enzymatic Hydrolysis

Glycosylases excise damaged nucleobases (for a review see Krokan and Standal 1997). They are grouped into 'simple glycosylases' that do not form DNA strand breaks and 'glycosylases/abasic site lyases' that concomitantly induce a stand break (Dodson and Lloyd 2002). This may have to be taken into account when using these enzymes for the detection of damaged sites (for the use of endonucleases for fingerprinting of DNA damage, see Epe and Hegler 1994).

As mentioned briefly above, the enzymatic excision of damaged nucleobases may cause some problems. A case in point is the action of nuclease P1. While a single 8-oxo-G lesion is excised as the damaged nucleoside, the clustered 8-oxo-G/Fo lesion is only obtained as modified dinucleotide (Maccubbin et al. 1992). Another example is the hydrolysis of dG*pC which severely inhibits the action of bovine spleen phosphodiesterase, while HMUrapA shows only very little inhibition (Maccubbin et al. 1991). Enzymatic hydrolysis of DNA is, in fact, the recommended method for the determination of HMUra (Teebor et al. 1984; Frenkel et al. 1985). It is recalled that mammalian cells cope with this DNA lesion with the help of a hydroxymethyluracil glycosylase (Hollstein et al. 1984).

When the Tg lesions is opened by ammonolysis, the resulting product (α-*R*-hydroxy-β-ureidoisobutyric acid) inhibits snake venom phosphodiesterase, λ exonuclease and the Klenow (exo$^-$) fragment (Matray et al. 1995; see also Greenberg and Matray 1997). It is, however, removed by *E. coli* Fpg and Nth proteins (Jurado et al. 1998).

A detailed study on the repair of the cA lesion is available (Brooks et al. 2000). The enzymatic excision of 8-oxo-A by Ogg1 from *S. cerevisiae* is only effective when this damage is paired with Cyt (but not Ade, Thy, Gua or Ura; Girard et al. 1998; for a review on the action of this enzyme in excising 8-oxo-G see Nishimura 2002). Substantial neighboring effects are also observed for the excision of other lesions such as 8-oxo-G or AP sites. Excision of 8-oxo-G by the *E. coli* Fpg protein is used as the first step for an improved detection of this lesion (Beckman et al. 2000; ESCODD 2003). There is a large variation in the yields of 8-oxo-G and concomitant discussions as to the best method for the detection of this DNA lesion. Attention has been drawn that incomplete digestion of the damaged DNA by the enzymatic cocktails typically used may be one of the reasons for such discrepancies, and an improved protocol has been suggested (Huang et al. 2001).

The development in this area of enzymatic action on the various damaged DNA sites continues to be very active. For this reason, only a very short account has been given as a kind of flavor for the reader to see in which direction research in this field seems to expand.

13.2.3 Detection of DNA Lesions by Gas Chromatography/Mass Spectrometry

Most of our present knowledge of free-radical-induced DNA lesions is based upon their identification and often also quantification by GC/MS. In order to convert the nucleobases and their free-radical-induced products into sufficiently volatile compounds the -NRH and -OH groups have to be trimethylsilylated. Carbohydrate-type products resulting from an alteration of the sugar moiety may be reduced with $NaBH_4$ after release or excision from DNA into the corresponding polyhydric alcohols (Beesk et al. 1979). Reduction with $NaBD_4$ incorporates a deuterium atom at the position of a carbonyl function (two deuterium atoms at a carboxyl group). The mass spectra of the trimethylsilylated polyhydric alcohols usually allows a firm assignment of their structure when the number of carbon atoms is known from the GC retention time (Dizdaroglu et al. 1974). A methoximation of the carbonyl function in combination with a trimethylsilylation of the hydroxyl groups also converts carbohydrate products into volatile compounds, and their mass spectra may provide additional information (Dizdaroglu et al. 1977). For the determination of the carbohydrate products, a polyhydric alcohol that is not formed under the given conditions can be used as internal standard. For the quantification of the base products, isotopically-labeled reference material which also undergoes the various prepurification steps (e.g., by semi-preparative HPLC) may be added (Bianchini et al. 1996; Douki et al. 1996; D'Ham et al. 1998). The determination of altered bases by GC/MS-SIM (SIM = single-ion monitoring), after trimethylsilylation, has become the standard method for

their determination (Dizdaroglu 1985, 1990, 1991, 1992; Dizdaroglu and Bergtold 1986; Fuciarelli et al. 1989; Dizdaroglu and Gajewski 1990; Jaruga et al. 2001).

13.2.4 Determination of DNA Lesions by Liquid Chromatography

When the term 'liquid chromatography' is used these days, it usually refers to HPLC (for reviews see Zakaria and Brown 1981; Scoble and Brown 1983), but valuable separations can also be achieved by TLC (Cadet et al. 1983). Although detection in HPLC is mainly by UV spectrophotometry (e.g., Cadet et al. 1982), electrochemical detection is the method of choice in the case of the strongly reducing 8-oxo-G lesion (Floyd et al. 1986; Shigenaga et al. 1989, 1990; Berger et al. 1990; Mei et al. 2003). In a multi-laboratory test, this method proved to be much more reliable than GC/MS and HPLC/MS-MS methods (ESCODD 2003). The work-up of biological samples and the difficulties that one might encounter have been discussed (Claycamp and Ho 1993; Douki et al. 1997; Dany et al. 1999). This is of importance, since this compound is often used as a marker of oxidative DNA damage, for example, in assessing the role of dietary fats (Loft et al. 1998).

Electrochemical detection is also the method of choice for the reducing dCyd-derived products 5OHdCyd and 5OHdUrd that can be released enzymatically from oxidized DNA (Wagner et al. 2004). After water elimination, the Ug may also be determined by this technique.

The sugar lesions FUR and 5-MF (Joshi and Ganesh 1994), as well as DNA-adducts (Park et al. 1989) have also be detected with the help of HPLC.

For optical detection, the product to be determined must have a reasonable absorption coefficient. Saturation of the pyrimidine *C*(5)-*C*(6) bond destroys the chromophore, and for the determination of Tg yields excreted into the urine, this product was reduced with HI into Thy prior to an HPLC analysis (Cathcart et al. 1984).

The combination of HPLC and MS (cf. Frelon et al. 2000) is expected to become the method of choice in many cases. The state of the art has been reviewed (Cadet et al. 2002).

13.2.5 MALDI-TOF

Another very sensitive MS technique is MALDI-TOF. Its high-resolution mass spectra allowed, for example, the identification of the 8-oxo-G lesion by its +16-Da mass shift in a oligonucleotide heptamer at picomol level (Bartolini and Johnston 2000).

13.2.6 Capillary and Pulsed Field Gel Electrophoresis

Capillary gel electrophoresis (CGE) has the advantage of a short analysis time, high degree of automation and reproducibility combined with a good resolving power for dsDNA fragments. The mobility of a DNA fragment is given by equa-

tion (6), where V is the steady-state velocity of the fragment, k a factor related the net polyanion charge Q, S the size of the fragment (also related to Q), E the electrical field, E_a the activation energy for the viscous flow, R the universal gas constant and T the absolute temperature.

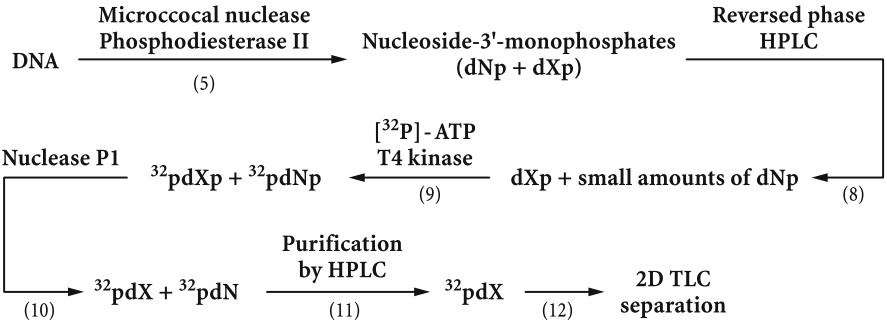
$$\ln \frac{1}{V} = \ln \frac{k}{ES} + \frac{E_a}{RT} \tag{6}$$

As an example for the study of DNA damage after irradiation using this technique may serve reference (Valenzuela et al. 2000).

In pulsed field gel electrophoresis (PFGE), intact DNA is treated with restriction enzymes to generate pieces small enough to resolve by electrophoresis in an agarose or acrylamide gel. With each reorientation of the electric field relative to the gel, small-sized DNA will begin moving in the new direction more quickly than the larger DNA. Thus, the larger DNA lags behind providing a separation from the smaller DNA (for a review see Anand and Southern 1990). Among others, PFGE seems to be the most sensitive technique for the determination of DSBs in cells (Rothkamm and Löbrich 2003).

13.2.7 ^{32}P-Postlabeling

The ^{32}P-postlabeling technique allows to improve the sensitivity of the detection of DNA damage (Cadet et al. 1998). The damaged DNA is enzymatically degraded into nucleotide-3-phosphates [reaction (7)]. The resulting mixture of unchanged nucleoside-3-phosphates (dNp) and damaged ones (dXP) are separated by HPLC [reaction (8)]. They are then labeled at the 5′-position with ^{32}P [reaction (9)] and subsequently dephosphorylated at the 3′-position [reaction (10)]. This allows to proceed with a second purification and their identification by, for example, two-dimensional TLC [reactions (11) and (12)].



Factors that affect the determination of 8-oxo-G by this technique have been discussed in some detail (Möller et al. 1998). The determination of Tg by this technique (Hegi et al. 1989) is one of its most sensitive assays (Weinfeld and Soderlind 1991), many orders of magnitude higher than the earlier determination by HPLC (Frenkel et al. 1981). A ^{32}P-postlabeling assay for the cA lesion which blocks gene

expression and is repaired by the nucleotide excision repair pathway (Brooks et al. 2000) has also been developed for the use in mammalian tissues (Randerath et al. 2001). Ade-*N*-1-oxide, a product that is formed when DNA is exposed to H_2O_2, has also been determined using this technique (Mouret et al. 1990).

13.2.8 Detection of Hydroperoxides

Hydrogen peroxide and organic hydroperoxides can be reduced by molybdate-activated iodide [Allen et al. 1952; reactions (13) and (14)]. The iodine atoms formed in these reactions combine to iodine which complexes with iodide [reactions (15) and (16)], and it is this I_3^- complex which is measured (ε(3540 nm) = 25,000 $dm^3\ mol^{-1}\ cm^{-1}$)

$$ROOH + I^- \rightarrow RO^\bullet + I^\bullet + OH^- \quad (13)$$

$$RO^\bullet + I^- \rightarrow RO^- + I^\bullet \quad (14)$$

$$2\,I^\bullet \rightarrow I_2 \quad (15)$$

$$I_2 + I^- \rightarrow I_3^- \quad (16)$$

Hydrogen peroxide reacts considerably faster with this reagent ($t_{1/2}$ = 2.5 s) than organic hydroperoxides, and the differences in the rate of buildup not only allows to determine H_2O_2 and organic hydroperoxides side by side, but often the rate of reaction of a given hydroperoxide with this reagent can be used as a fingerprint for its presence (Dowideit and von Sonntag 1998; Flyunt et al. 2003). A certain disadvantage of this reagent is the fact that the iodine thus formed may react with other products (Al-Sheikhly et al. 1984). This problem can be overcome in certain cases by combining it with an HPLC separation (post-column derivatization mode). For a quantification of a hydroperoxidic product by this method the slowness of reaction of some hydroperoxides with this reagent may require the heating of the reaction coil, but in unfavorable cases the reaction may still remain incomplete. This may result in an underestimate of the hydroperoxide yield.

Hydroperoxides also react readily with Fe^{2+} in acid media [reactions (17) and (18)]. The Fe^{3+} formed can be complexed with xylenol orange. This complex has a strong absorption coefficient in the visible (ε(540 nm) = 26,800 $dm^3\ mol^{-1}\ cm^{-1}$). This method has been used with some advantage in the identification of the hydroperoxides formed upon $^\bullet$OH-attack on Thd by HPLC using the post-column derivatization technique (reagent: e.g. 2.3×10^{-4} mol dm^{-3} ammonium ferrous sulfate, 3.5×10^{-2} mol dm^{-3} sulfuric acid, 5.6×10^{-4} mol dm^{-3} xylenol orange; Wagner et al. 1990).

$$ROOH + Fe^{2+} \rightarrow RO^\bullet + OH^- + Fe^{3+} \quad (17)$$

$$RO^\bullet + Fe^{2+} \rightarrow RO^- + Fe^{3+} \quad (18)$$

As in the related Fricke actinometer, there is a given chance that more than two Fe^{3+} are formed, and using the Fe^{2+}/xylenol orange system hydroperoxide yields may be overestimated as has been shown for the Fe^{2+}/SCN^- system (Mihaljevic et al. 1996). The alkoxyl radical, besides being reduced by Fe^{2+} [reaction (18)], may undergo rapid β-fragmentation (Chap. 7.2) and the alkyl radical thus formed adds O_2, yielding a peroxyl radical which can be reduced by Fe^{2+} to the corresponding hydroperoxide. This sequence is a chain reaction, only interrupted by the reduction of the alkoxyl radical [reaction (18)]. In the Fricke actinometer Cl^- can be added which converts $^{\bullet}OH$ into $Cl_2^{\bullet -}$. This radical reacts readily with Fe^{2+} but does not continue the chain reaction by reacting with organic impurities. In the above assay, the addition of Cl^- will not have a beneficial effect, because alkoxyl radicals do not oxidize Cl^- in acid solutions.

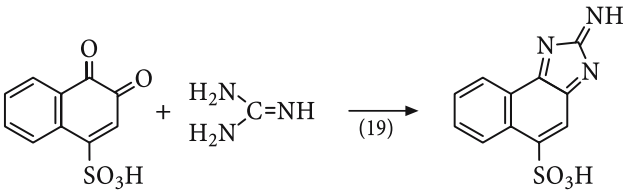
13.2.9
Malonaldehyde, Base Propenals and Related Compounds

Malonaldehyde and related compounds react readily with 2-thiobarbituric acid (TBA, typically 2 g dm^{-3}, the resulting pH of the solution is thus around 2, at 70 °C for several minutes, depending on the reactivity of the compound to be analyzed) and turns yielding a pink color (in the case of malonaldehyde ε(532 nm) = 150,000 dm^3 mol^{-1} cm^{-1}). The rate of reaction of the base propenals is faster than that of malonaldehyde (Rashid et al. 1999), i.e., they do not hydrolyze prior to their reaction with TBA. The reaction of the 5′-aldehydes derived from Pur deoxynucleosides react more slowly (Langfinger and von Sonntag 1985; von Sonntag 1994), and the color tends to fade. Other lesions damaged sugar moiety also give a positive TBA response, for example, 3′-oxo-dThd (Rashid et al. 1999) and unknown DNA-bound damaged sites (Krushinskaya and Shalnov 1967; Krushinskaya 1983; Rashid et al. 1999). The fact that there are definitely different products formed in the TBA reaction, although they all give rise to the same kind of absorption raises the question, whether the same absorption coefficient as has been determined for malonaldehyde can also be used for the other TBA-reactive compounds. With dGuo-5′-aldehyde, this is clearly not the case (Langfinger and von Sonntag 1985).

To avoid some of the ambiguities of the common TBA assay described above, malonaldehyde may be converted with methylhydrazine under very mild conditions into 1-methylpyrazol. The latter is extracted and determined by GC (Matsufuji and Shibamoto 2004).

13.2.10
Guanidine Derivatives

Some Gua lesions are characterized by a guanidine residue that can be detected spectrophotometrically using 1,2-naphthoquinone-4-suphonate as the reagent (Kobayashi et al. 1987). The resulting product is shown in reaction (19).



13.2.11 Immuno Assays

Immuno assays have been developed for a variety of DNA lesion such as UV-damage (McConlogue et al. 1982; Eggset et al. 1987), Tg (Rajagopalan et al. 1984; Le et al. 1998) cA (Fuciarelli et al. 1985, 1987), Ade-N^1-oxide (Signorini et al. 1998), carcinogen-modified DNA (Müller et al. 1982), hydroxylation of guanine (Kasai and Nishimura 1986), 8-oxo-G (Yin et al. 1995), HMdUra (Lewis et al. 1978), DNA base damage (Lewis and Ward 1978) and X-ray-induced damage (Waller et al. 1981).

A fluorescent 90-mer oligonucleotide in combination with laser-induced fluorescence and capillary electrophoresis has been used as a versatile probe for a detection of DNA damage (Carnelley et al. 2001).

13.2.12 Atomic Force Microscopy

Atomic force microscopy has been developed to a stage that DSBs and the length of the resulting fragments can be detected by this technique (Pang et al. 1996). In this context it is of interest that neutron irradiation leads to the formation of very small fragments (Pang et al. 1997).

13.2.13 Comet Assay

The comet assay (Ostling and Johanson 1984) makes use of the fact that the smaller DNA fractions that are formed when the molecular weight of the cellular DNA is reduced by free-radical-induced DSBs move faster in an applied electric field (after cell lysis) than the undamaged DNA. Staining the DNA yields a comet-like pattern. Purified repair enzymes, applied to DNA during the course of the comet assay procedure, can greatly enhance the sensitivity and specificity of the assay (Collins et al. 1997; Sauvaigo et al. 2002). This assay is now widely applied (cf. Pouget et al. 1999), and a combination with immunofluorescence detection techniques allows even the detection specific damage and its repair (Sauvaigo et al. 1998). For the application of the comet assay in assessing environmental hazards, see Ündeger et al. (1999), for a comparison of γ-irradiated and BLM-treated cells, see Östling and Johanson (1987).

Table 13.1. Enzymes used for the identification of clustered DNA base damage. (Sutherland et al. 2000)

Enzyme	Class recognized	Lesion recognized
E. coli Fpg protein (formamidopyrimidine-DNA glycosylase)	Oxidized purines	FAPY-A, FAPY-G, 8-oxo-G, 8-oxo-A, some abasic sites, to a lesser extent other modified purines
E. coli Nth protein (endonuclease III)	Oxidized pyrimidines	Ring-saturated or fragmented Thy residues, e.g. H_2Thy, Tg, 5-hydroxy-5-methylhydantoine, urea, DNA damaged at Gua, some abasic sites
E. coli Nfo protein (endonuclease IV)	AP sites	Several AP sites including oxidized AP sites, urea

13.2.14 Detection of SSBs and DSBs with the Help of Supercoiled Plasmids

Plasmids are small pieces of circular supercoiled dsDNA. A SSB causes the plasmid to relax into the open circular form, a DSB into the ds linear form. These there forms can be separated by chromatographic methods (e.g., Bresler et al. 1979). This assay is widely used for studying effects of various agents including the action of enzymes on damaged DNA.

13.2.15 Detection of Clustered Lesions

DNA damage induced by ionizing radiation leads single lesions also to the formation of clustered lesions such as two close-by damaged bases on opposite strands (Chap. 12). For their detection, DNA is treated with an endonuclease that induces a SSB at a damaged site. If there are two closely separated lesions on opposite strands, such treatment induces a DSB which can be detected on a non-denaturing gel (Sutherland et al. 2000). The enzymes that have been used and their targets are compiled in Table 13.1.

13.3 Pulse Radiolysis and Laser Flash Photolysis

The pulse radiolysis technique is close to the better known laser flash photolysis (for a monograph see Bensasson et al. 1983). There is one essential difference: in pulse radiolysis the energy is absorbed by the solvent, e.g., by water in DNA solu-

tions, while in laser flash photolysis the energy of the photons is absorbed by the solute, e.g., by DNA. Although at high enough photon energies such as 193 nm or upon biphotonic excitation ionization does occur yielding radical cations and electrons, i.e. DNA radicals are formed upon UV-excitation, the application of laser flash photolysis in the study of DNA free-radical chemistry has found few applications so far. This is, however, changing as more and more photolabile compounds tailored to produce specific radicals are studied. In both techniques, the detection systems are the same, and the evaluation of the data follow the same principles. Some basics of these techniques will be described now.

The key reactions of the radiolysis of water leading to the formation of $^{\bullet}OH$, e_{aq}^- and $H^{\bullet}$ and the conversion of e_{aq}^- into a further $^{\bullet}OH$ have been discussed in Chapter 2.2. Here, it is sufficient to recall that the spur reactions are over in ca. 10^{-8} s, and from thereon the distribution of the radical species is practically homogeneous.

Short electron pulses are produced using Van de Graaff, LINAC or Febetron-type accelerators. Detection of products (intermediates) that grow in and decay as the cascade of reactions initiated by the pulse proceeds may be based on optical absorption, optical emission, electrical conductivity, EPR, CIDNP, polarography and light scattering. When pulse radiolysis is combined with a rapid-mixing device, the reactions of long-lived radicals with added substrates can also be studied (see below). Opaque solid DNA sample can also be investigated using the diffuse-reflectance technique (Wilkinson et al. 1984; O'Neill et al. 1989). Different types of pulse-generating and detection equipment have been described in the literature (Barker et al. 1970; Warman et al. 1980; Asmus and Janata 1982a,b; Janata 1982, 1992a,b; van Lith et al. 1983; Asmus 1984; Tabata et al. 1985; Patterson 1987; von Sonntag 1989; Janata et al. 1993; Bothe and Janata 1994; von Sonntag and Schuchmann 1994).

In a typical pulse radiolysis experiment, a submicrosecond pulse of high-energy electrons (2.5–10 MeV) passes through the cell containing the system to be investigated. Upon losing its energy, a 3-MeV electron (penetration depth in water ca. 10 mm) gives rise to as many as 1.8×10^8 radicals ($^{\bullet}OH$, e_{aq}^- and $H^{\bullet}$) together with further charged particles (H^+ and OH^-) after completion of the spur reactions (Chap. 2.2). In pulse radiolysis, the number of primary radicals formed does not depend on the properties of the solute and its concentration. In laser flash photolysis, however, the substrate concentration and the absorption coefficient at the given excitation wavelength but also on the quantum yield of radical formation determine the efficiency of radical formation. The latter may often be the limiting factor for using this technique. Moreover in laser flash photolysis, detected intermediates are usually not only due to free radicals, but triplet states or non-radical products resulting from the reactions of the excited states may give rise to the observed effects as well.

Intermediates may be detected by UV-Vis spectroscopy. In a pulse radiolysis experiments, Čerenkov light is emitted as the high-energy electrons are slowed down. Hence, when the photon flux of the analyzing light (I_0) is low, emission signals are observed despite the fact that absorbing species are formed (in laser flash-photolysis this phenomenon is only observed if the quantum yield of fluorescence is high and the monitoring wavelength is set in this wavelength region).

The problem can be overcome by boosting for a few milliseconds the power of the lamp providing the analyzing light (with a 450-W xenon lamp about tenfold). Thus, the I_0 value of the analyzing light is now much higher than the intensity of the Čerenkov light (fluorescence) and signals reflect with sufficient accuracy the absorption properties of the intermediates formed during the pulse (laser flash). Recently, attention has been drawn to pitfalls by stray light (Czapski et al. 2005) and solute absobance (especially in laser flash photolysis; von Sonntag 1999). Data acquisition and storage are computerized which allows multiple-signal averaging. The dose in a pulse radiolysis experiment may be determined (Butler and Land 1996) by the thiocyanate dosimeter (Schuler et al. 1981), more recently revised (Buxton and Stuart 1995; $G(SCN)_2^{\bullet-} \times \varepsilon_{475\,nm} = (2.59 \pm 0.05) \times 10^{-4}\ m^2\ J^{-1}$).

In many free-radical reactions, neutral radicals give rise to charged species. For example, neutral peroxyl radicals may release $HO_2^\bullet/O_2^{\bullet-}$ ($pK_a(HO_2^\bullet) = 4.8$; Chap. 8.11). The equivalence conductance of H^+ and OH^- is 315 and 175 Ω^{-1} mol^{-1} cm^{-1}, respectively. Monoanions and monocations have values in the range of 45-60 Ω^{-1} mol^{-1} cm^{-1}. When the neutralization is completed, the signal of the change of conductance produced by the charged species will be large and positive at pH below pH 7 [expression (20)], but smaller and negative above pH 7 [expression (21)]. The neutralization reaction must not push the pH out of the basic range. The lowest pH at which an experiment can be carried out in basic solution is hence approximately pH 9.

Acid solution:

$$\underline{\text{Pulse} \rightarrow H^+ + X^-} = +315 + 45\ \Omega^{-1}\ \underline{\text{mol}^{-1}\ \text{cm}^{-1}} \qquad (20)$$
$$\Delta\kappa = +360\ \Omega^{-1}\ \text{mol}^{-1}\ \text{cm}^{-1}$$

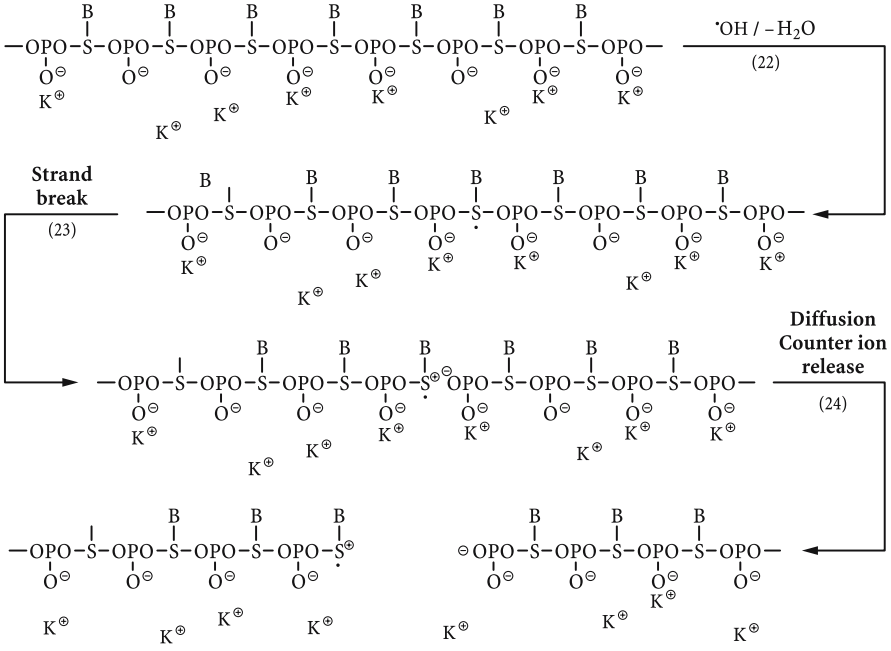
Basic solution:

$$\text{Pulse} \rightarrow H^+ + X^- = +315 + 45\ \Omega^{-1}\ \text{mol}^{-1}\ \text{cm}^{-1} \qquad (21)$$
$$\text{Neutralization: } H^+ + OH^- \rightarrow H_2O = -315 - 170\ \Omega^{-1}\ \text{mol}^{-1}\ \text{cm}^{-1}$$
$$\Delta\kappa = -125\ \Omega^{-1}\ \text{mol}^{-1}\ \text{cm}^{-1}$$

The available set-ups are very sensitive, and a pH range between 2.5 and 12 is accessible. Obviously, the presence of buffers will have a considerable effect on the signal height, but in favorable cases a computer analysis may allow the quantification of the various contributions to the conductance signal even under such conditions (Das et al. 1987; Schuchmann et al. 1989).

For dosimetry, the reaction of the reaction of $^\bullet$OH with DMSO which yields methanesulfinic acid (92%; Veltwisch et al. 1980; Chap. 3.2) is usually used. This allows one to put the conductance signals on a quantitative basis (calculation of G values), and the rates of reactions that are kinetically of first order can be determined for the time dependence of the signal evolution. DMSO dosimetry yields only a relative dose. For the determination of second-order rate constants, however, the exact dose must be known, and this can be determined by the 'zero conductivity change dosimetry' or 'neutralization kinetics dosimetry' (Schuchmann et al. 1991).

The application of the conductance changes has been of considerable importance in assessing the kinetics of free-radical-induced chain scission of charged polymers (Chap. 9.4). A polymeric electrolyte exerts a strong electric field in the vicinity of the polymer chain. This cause counterions to "condense" onto the polymer. When in single-stranded DNA, for example, •OH attacks the sugar moiety at, let us say at C(4′) [reaction (22), cf. Chap. 12.4], the number of condensed counterions does not change, nor at the very moment when the strand breaks upon the heterolytic cleavage of the phosphate bond [reaction (23)]. However, when the two ends diffuse apart, condensed counterions (*e.g.*, K^+) ions are released [reaction (24)]. This causes a conductance increase. Insofar as this process does not involve the formation of H^+, the conductance change signal remains positive even in an alkaline environment, since a neutralization reaction does not intervene. As long as the rate of the diffusive process (24) is fast compared to the preceding reactions (22) and (23), the observed kinetics are those of strand breakage [reaction (23); the •OH reaction (22) is never rate-determining]. In all the systems investigated thus far, this requirement has always been met.



Detection by laser light-scattering (LLS) is another means of determining changes in the molecular weight of polymers. This method is, of course, not restricted to charged polymers. LLS detection can be carried out at the 90° angle mode (Schnabel 1986 and references cited therein) but also at the low-angle mode (~ 7°, LALLS). The advantage of LLS is that the setup is simpler, and a better signal-to-noise ratio is obtained, but problems arising from form factors are minimized with LALLS. Intermolecular cross-linking gives rise to an increase chain scission to a decrease in signal intensity. Upon folding of a rod-like polymer the LSI

signal is also increased. Thus, in such a case, intramolecular cross-linking can also contribute to an increase in the LSI signal.

Although the signal-to-noise ratio in the case of the LALLS technique is much poorer than in the case of conductance method, one may have to use LALLS also in the case of charged polymers, whenever other sources of conductance changes are expected (e.g., release of $HO_2^{\bullet}/O_2^{\bullet -}$ in peroxyl radical reactions; Chap. 8.4).

The volume of activation which can provide very valuable information concerning the reaction mechanism is obtained by high-pressure pulse radiolysis (for a review in the area of transition metal ion chemistry, see van Eldik and Meyerstein 2000).

Single charged-particle beam irradiation of single cells has been developed to study various aspects of radiation biology such as the bystander effect. This subject exceeds the scope of this book, and only some reference to this technique is made here (Folkard et al. 1997a,b).

13.4 Gas Explosion and Rapid-Mixing Techniques

The gas explosion technique (Michael et al. 1973) allows one to change on the sub-millisecond time scale the environment of a target layer of plasmid DNA or cells by letting a burst of gases, e.g., O_2 or H_2S, enter the irradiation chamber at times before or after a short pulse of radiation has been given to the target. The gas burst is triggered by a solenoid valve, and the gas under pressure proceeds to the target at a typical speed of 70 m s^{-1}.

Another way of changing the environment or adding a reactant at a given time before or after a short pulse of radiation is the rapid mixing technique (e.g., Bielski and Richter 1977). In a stopped-flow set-up, long-lived radicals such as $O_2^{\bullet -}$ that have been generated from a solution contained in one syringe in the pulse-irradiated zone are reacted by mixing with a substrate contained in another syringe. Distortions of the kinetics are prevented by a hard stop. The typical rise time of such a set-up used to be 10 ms, but advanced techniques now provide a rise time of about 1 ms.

With some modifications, such a technique has been used to study the time scale of the effects of O_2 and sensitizers on cells in aqueous solution (Adams et al. 1968; Whillans 1982; Whillans and Hunt 1982). The rapid lysis technique works on the same principle. It permits cells to be lysed at a given time after a dose of radiation (Johansen and Boye 1975; Johansen et al. 1975; Sapora et al. 1975, 1977; Fox et al. 1976; Millar et al. 1980). This allows the study of repair kinetics at early times.

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